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Comparative Agonistic and Feeding Behavior of Hatchery-Reared and Wild Salmon in Aquaria[1,2,3]

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ABSTRACT

When hatchery-reared and wild landlocked Atlantic salmon (*Salmo salar*) parr of the same age and size were permitted to compete for social dominance and for food in aquaria, twice as many hatchery salmon attained dominance as wild salmon. Dominant hatchery salmon also showed a higher intensity of aggressiveness than dominant wild salmon, displaying a higher and more variable mean nipping rate. Socially dominant salmon ate more food per fish than subordinates, but there were no statistically significant differences in feeding rate between dominant hatchery and dominant wild salmon, or between subordinate hatchery and subordinate wild salmon.

Hatchery salmon displayed lower feeding rates than wild salmon when they were held in separate compartments of an aquarium and compared at three temperatures. This difference in feeding rate probably was not a reflection of differences in adaptation to temperature or food preference, but, rather, was the result of interference with feeding caused by the more intense social interaction among hatchery fish.

It is suggested that high levels of aggressiveness may contribute to mortalities of hatchery-reared salmon planted in streams because of loss of feeding time, excessive use of energy, and increased exposure to predators.

INTRODUCTION

THE MAINE Department of Inland Fisheries and Game has undertaken a comprehensive investigation of the comparative behavior of juvenile hatchery-reared and wild landlocked Atlantic salmon (*Salmo salar* L.). A major objective of this investigation is to determine if hatchery salmon exhibit behavioral aberrations or deficiencies that may lower their chances for survival when released in the wild. Reported here are the results of two experiments conducted during the winter of 1964–65 at the Roland H. Cobb Fish Cultural

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Station, Enfield, Maine, for the purpose of comparing the social and feeding behavior of hatchery and wild salmon parr under controlled conditions.

Young Atlantic salmon are commonly referred to as *parr* during their years of stream residence, a term distinguishing them from the *smolt* stage, which they usually enter in the 2nd or 3rd year of life just before emigration. Salmon parr are territorial and defend an area of the stream substrate around a localized feeding station. In defense of the territory, the parr display stereotyped threat postures and aggressive or submissive acts toward adversaries. They also exhibit color changes which are indicative of their social status and success in winning territorial or hierarchial disputes. Their movements are generally restricted to the immediate area of the feeding station, from which they make short excursions for food materials which drift near them in the current. The social and feeding behavior of Atlantic salmon have been described by Kalleberg (1958), who studied them in a stream aquarium simulating natural conditions. Essentially similar behavior has also been described by Keenleyside and Yamamoto (1962), who studied juvenile Atlantic salmon in laboratory aquaria. Both studies were concerned with the behavior of anadromous salmon. Judging from these descriptions and our own observations, there are no important differences in the social and feeding behavior of juvenile sea-run and juvenile landlocked Atlantic salmon.

Because agonistic behavior associated with territoriality is an important component of the salmon's behavior during its stream life, and must be of survival value in competition for food and space, the first of the experiments described was designed to determine if hatchery salmon are capable of competing with wild salmon for social dominance and natural food. Because summer and winter water temperatures are controlled and held nearly constant at the Cobb Station and because such constancy does not occur in natural streams, a second experiment was conducted to determine if feeding behavior of hatchery and wild salmon is affected differently by different temperatures. Although the second experiment was designed simply to compare feeding responses of two groups of fish, it became clear as the experiment progressed that social behavior would also influence its results.

The planned use of aquatic insects as experimental food raised the question of whether or not hatchery salmon reared on beef liver would show the same acceptance of natural food as the wild salmon. Both experiments allowed a comparison of the acceptance of natural food by hatchery-reared and wild salmon.

Because salmon reared in Maine hatcheries are progeny from wild parents, it is likely that any behavioral differences between salmon of wild and hatchery origin are environmental effects rather than the results of genetic change through domestication, as may have occurred with domestic strains of brook trout (Vincent, 1960). Smith (1961) suggests that even short-term exposure to the hatchery environment may alter the ability of fish to adapt to the natural environment and thus lessen their chances for survival.

GENERAL METHODS AND MATERIALS

Experiments were conducted in adjacent aquaria located at one corner of the hatchery building. For the experiment on social behavior, two 125-liter plexiglas aquaria were used, each measuring 61 × 46 × 46 cm. Water from the hatchery water supply (lake water) was run continuously through each aquarium at about 2 liters/min from a 26-mm plastic pipe angled over the side at one corner. Approximately 25 mm of gravel was placed on the bottom of each aquarium, and the water depth was maintained at 36 cm above the substrate by an overflow outlet near the top of the wall opposite from the intake. A slight current was set up in the aquaria, and there was some tendency for salmon resting near the intake corner to orient to the flow coming from the pipe. To eliminate outside visual disturbances, three sides of each aquarium were faced with opaque paper, and the clear side was covered with a cardboard box provided with narrow slits for observation. A glass feeding tube extended from the water surface at the center of each aquarium through a translucent cover to a point over the observation box where the observer could reach it without disturbing the fish by overhead movement. Light for the aquaria was provided by natural illumination through windows and 100-w incandescent ceiling lamps.

For the experiment on feeding response to temperature, a 181-liter aquarium measuring 122 × 46 × 41 cm and constructed of fiberglased plywood with one glass wall was used. A plywood center partition divided the aquarium into two equal compartments. The partition was perforated by 3-mm holes spaced 25 mm apart to allow circulation of water but still maintain effective visual isolation between compartments. There was no indication that fish in one compartment were aware of fish in the other compartment during tests. The bottom was covered with 25 mm of gravel. Each compartment contained an aerator-filter and an electric heating element which controlled water temperature within ±0.5 C. The fish were tested at 3, 12, and 19 C. At the high temperature it was necessary to add small amounts of cold water to replace evaporation loss. At the low temperature the heating elements and pumps were turned off, and water from the hatchery supply was run through the aquarium at about 2 liters/min, flowing in at the top of one end of the aquarium in one compartment and out the other. Water depth was held at about 35 cm. In this aquarium there was no observable tendency for fish in either compartment to orient to a slight circular current. Cover for the fish was provided in each compartment by four 50 × 50 × 100-mm open-ended aluminum boxes, painted olive drab and placed on the bottom with their entrances oriented in the direction of the feeding tubes. A viewing box was placed over the window, and each compartment was outfitted with a feeding tube. Additional light for the aquarium was provided by two table lamps with 100-w incandescent bulbs which were set atop a translucent glass cover.

The hatchery and wild salmon used in these experiments were parr of approximately the same age (10–12 months). Hatchery salmon were dipped from an outdoor raceway as needed for successive trials of an experiment.

On the same day hatchery salmon were obtained, wild salmon were obtained with a pulsed d-c electrofishing unit from Cold Stream, the outlet of the lake which furnished the hatchery water supply. All available evidence indicates no lasting effect of electrofishing on the behavior of the fish when the equipment is used properly. When it was necessary for the wild salmon to be held before a trial, they and their hatchery-reared counterparts were kept in an aluminum rearing trough measuring 264 × 46 × 36 cm. They were not fed during this time. Before each trial each fish was anesthetized with M.S. 222, measured, and given a distinctive mark by clipping the adipose fin or small portions of the caudal or dorsal fins. There was no indication that this slight marking of the fins affected the behavior of the parr.

Live mayfly (Ephemeroptera) and stonefly (Plecoptera) nymphs were used as experimental food because they are a natural food of young salmon and because their swimming movements keep them from settling to the substrate too rapidly. This movement attracted the attention of the salmon and made the food available for a suitable length of time. The nymphs, as determined by prior experiments, were of a size acceptable to the small salmon. Nymphs not eaten during their descent were rarely eaten after reaching the substrate. The food was introduced by drawing each insect into an eye-dropper with water and flushing it down the feeding tube.

Although routine operation of the hatchery was going on in the room, only on one occasion during the experiment on social behavior was there noticeable disturbance to fish during an observation period. On that day unusually loud noise was created by hatchery personnel doing repair work, and the experiment had to be delayed until the next day.

All statistical hypotheses in this report were evaluated with a maximum probability of chance rejection of $p \leq 0.05$.

SOCIAL AND FEEDING BEHAVIOR OF HATCHERY AND WILD SALMON

PROCEDURE

This experiment consisted of 10 trials, 5 in each of the two aquaria located side-by-side in a corner of the hatchery building. Prior to the start of each trial, three wild and three hatchery-reared salmon parr were placed together in each aquarium and allowed to remain there undisturbed during a 36-hr acclimation period. Total lengths of the parr ranged between 74 and 105 mm, and were matched so that each wild parr had a hatchery-reared counterpart of the same length, within ±2 mm. All fish were marked as has been described, with the hatchery parr in one aquarium bearing the same marks as the wild parr in the other aquarium.

Each trial lasted 5–8 days, with each day's procedure consisting of three distinct operations: (1) feeding; (2) observation after feeding; and (3) removal of dominant fish. These operations were carried out by a single observer between 10:30 AM and 3:30 PM on consecutive days throughout the trial period, with one aquarium treated first on the 1st day, and the other aquarium treated first on the following day.

The food was introduced by flushing individual mayfly or stonefly nymphs down through the feeding tube one-at-a-time until the number of nymphs fed was equal to three times the number of parr in the aquarium. No nymph was introduced until the previous one had been eaten or had escaped and settled to the substrate. The identity of each feeding fish and the number of nymphs it ate were recorded. A feeding session usually was completed in 5–30 min.

Each day's post-feeding observation period began immediately after the last nymph had been eaten or had escaped, and lasted for ½ hr. All agonistic activity taking place in the aquarium during this time was observed and recorded on a tape recorder, with special care taken to observe and record the identities of the fish who initiated, won, and lost the various aggressive encounters which took place. Aggressive activity was quantified by counting the nips delivered by each parr.

Whenever an identifiable order of dominance was established within an aquarium during the ½-hr observation period, the dominant fish was removed at the end of the period. If the dominance order had not been established or could not be recognized at the end of the observation period, all fish were left in the aquarium for an extra day's testing. In this way the number of fish in each aquarium was reduced from six to two over a period varying from 5 to 8 days. A trial was terminated after the dominance order was determined for the last two fish and they were removed from the aquarium.

General Observations

All agonistic behavior characteristic of juvenile Atlantic salmon were displayed by the parr in the aquaria. There appeared to be no qualitative differences between hatchery and wild parr. Agonistic elements included frontal and lateral threat displays, charging, chasing, nipping, fleeing, and color changes. These have been expertly described by Kalleberg (1958), and by Keenleyside and Yamamoto (1962). They will be described in less detail here.

In the frontal display, an aggressive parr fixes its eyes on an adversary and approaches slowly, its body arched with head lowered, all fins spread, mouth open slightly, and the floor of the mouth extended to form a rather conspicuous pouch. In its movement forward, it propels itself at an increasing rate with tail beats of small amplitude, going fastest as it nears the adversary. Just before reaching the target fish the aggressor opens its mouth wide and nips.

When the lateral threat is displayed, the posture of the fish has the appearance of alarm and a highly defensive attitude. All fins are extended to their maximum, the body is rigidly stretched with the head flexed upward, the tail lower than the head, and the opercules and floor of the mouth extended. During the display, the body performs a movement that has been likened to a spastic tremor. The body is propelled by quick, small-amplitude beats of the tail, which is opposed by a braking extension of the large pectoral fins. If the parr are mutually aggressive both may display laterally, circling each other

slowly, head to tail, or they may swim in parallel. Each fish tilts its body in a sagittal plane so that the dorsal surface is leaning toward and the ventral parts away from the adversary. The fish may tilt from one side to the other as they change position in relation to each other's body axis.

The frontal threat is of an offensive character, whereas the lateral threat is believed to be essentially a defensive act. The lateral threat appears most frequently in agonistic encounters between two mutually aggressive parr when dominance is in dispute, and is believed to be an external expression of opposing tendencies on the part of both antagonists to attack and flee at the same time (Kalleberg, 1958).

In a charge, the aggressive fish swims rapidly, dashing straight toward its adversary, often terminating the charge with a vigorous nip broadside or at the tail if the adversary takes evasive action. Although nips may be delivered frequently in extended battles, they seldom if ever result in anything more serious than a dislodgement of scales or slightly torn fins. If a parr submits to attack it will flee rapidly away. The aggressor may give up the attack after delivering a nip or continue to chase the submissive fish and nip repeatedly at its tail. Often the submissive fish, after a sudden burst of speed, glides to the substrate and assumes submissive posture and coloration. The parr then adducts the lobes of its caudal fin and presses its other fins close to its body. It crouches motionless on the bottom as if trying to be as inconspicuous as possible — even respiration may stop momentarily.

Color changes which are displayed by both the dominant and submissive fish are distinctive. The normal color patterns of the dominant parr fade so that it takes on a bleached appearance. In contrast, the subordinate parr takes on a distinctive mottled appearance of the dorsal parts, formed by the concentration of melanophores in its parr markings, which are contrasted with lightened interspaces and a light longitudinal band above the lateral line. Below the lateral line the normally distinct outlines of parr marks become blurred and the belly takes on a gray color. The sclera of the eye develops a dark, vertical band, and in extreme cases the sclera may turn totally black.

There was just enough space for one territory in the small aquaria used in our study. Under these confined conditions, the social structure formed among parr in the aquaria became a monarchy, rather than a mosaic of mutually defended territories as described by Kalleberg (1958). Within a day or two after being placed in an aquarium, one fish would establish dominance over all other fish. If this dominant was removed, another would take its place as the despot. Occasionally a type of hierarchy or *peck-dominance* would appear, as described by Greenberg (1947) for green sunfish (*Lepomis cyanellus*), whereby some individuals would dominate fish lower in the social hierarchy and occasionally challenge the dominant in fights. This relationship, however, tended to be suppressed by the overbearing dominant.

A social hierarchy was not always well established on the 1st observation day. On the 2nd day, however, the dominant parr was usually identified easily. The dominant parr, normal or pale in coloration, usually took up a

station on the substrate near the center of the aquarium. Other parr lay passively on the substrate around the perimeter of the aquarium or hovered near the surface. Some parr, particularly those that were under repeated attack by the dominant, would swim erratically against the walls in obvious attempts to escape. This was called "escape swimming" and is probably analogous to the exploratory behavior of Atlantic salmon in rectangular aquaria described by Keenleyside and Yamamoto (1962). Some parr performed the curious act of swimming with their snouts against a wall, at the same time jerking their heads from side to side and snapping their jaws. Even dominant parr engaged in bouts of escape swimming, indicating that all fish suffered from some degree of stress imposed by crowding. As time elapsed and the number of fish was reduced from six to two, escape swimming became less frequent. Both hatchery and wild parr displayed this behavior.

Many subordinate parr displayed submissive coloration during an entire observation period, indicating they were under continual social stress. Movements of subordinate parr near the substrate almost always elicited an attack by the dominant. The dominant parr would sometimes pursue a subordinate around the aquarium for a minute or more before settling back to its station or changing its attack to another subordinate. Extended chases sometimes started a chain reaction of escape movements by other parr.

At the low temperatures (2.2–3.3 C) prevailing during this experiment, the parr were generally less active than they would be at higher temperatures, and there was a greater tendency to maintain contact with the substrate. At higher temperatures a parr that is holding a territory may hover a few centimeters off the bottom. Under more natural conditions this fixation to the bottom is also under the influence of water current, which tends to restrict the station holding of a parr to direct contact with the substrate, depending on water velocity (Kalleberg, 1958).

Comparative Dominance of Hatchery and Wild Salmon

The number of times a hatchery or wild parr became dominant over other parr in the two aquaria is given in Table I. Surprisingly, 9 times out

Table I. Comparison of dominance frequencies between hatchery-reared and wild salmon in order of the removal of dominant fish from aquaria; 10 trials combined.

Sequence of dominant fish	No. of times		
	Hatchery-reared salmon	Wild salmon	Dominance undetermined
First	9	1	0
Second	6	4	0
Third	5	5	0
Fourth	6	3	1
Fifth	2	1	7
Lowest ranking fish	1	2	7

of 10 the first parr to become dominant was a hatchery-reared parr. When the first two dominants were removed the parr remaining were mostly wild, and agonistic activity was less likely to occur with the lower number of fish. In one trial the first, second, and third dominant parr were all hatchery fish, leaving three wild parr in the aquarium. The first and second dominant fish in five trials were also hatchery parr. This dominance succession did not occur with wild parr.

Hatchery parr were not only dominant more frequently than wild parr, but they exercised their dominance more vigorously. During 41 observation periods, dominant hatchery parr and dominant wild parr had mean nipping rates of 3.57 and 1.43 per fish, respectively; the variances of the means were 7.27 and 2.36 per fish, respectively. The difference between the variances was significant ($F = 3.08$, df = 12, 27). Since the variances were unequal, a "theoretical" t-test (Snedecor, 1956) was used to test for significant differences between the means. A significant t value of 3.24 (18 df) was obtained.

Food Apportionment

Dominant–subordinate relationships among the salmon parr had a decided effect on the apportionment of food. During 41 feeding sessions in the two aquaria, 405 out of 516 insects were consumed. Dominant parr accounted for 188, or 46% of the 405 insects eaten. An average of 3.4 subordinate parr consumed 217, or 54%. On an average then, a single dominant parr consumed a disproportionate amount of the food. An unequal nested factorial design tested by analysis of variance supported the conclusion that dominant parr consumed significantly more insects than subordinates ($F = 16.96$, df = 1, 78). There were no significant differences between the mean feeding rates of dominant parr from the two sources in the different trials ($F = 0.48$, df = 8, 70). Of 46% of the insects eaten by dominant parr, 37% were eaten by hatchery parr and 9% by wild parr; however, the small sample size of dominant wild parr precluded statistical significance. Combining data from all trials, there was no significant difference in the mean numbers of insects consumed by all subordinate parr when a wild or a hatchery fish was dominant ($t = 1.14$, 38 df), and no difference in the mean numbers of insects consumed by subordinate hatchery and wild parr ($t = 0.80$, 130 df).

Dominant parr tended to take up a station at the center of the aquarium where the food descended directly over them. Occasionally though, dominant parr would get less food than certain subordinates that took advantage of the situation. Some subordinates which hovered at the surface near the feeding tube consumed nearly all the insects before they had a chance to drop near the substrate. On other occasions, subordinates took advantage of a reprieve from attack to get food while the dominant was preoccupied with an extended chase of another subordinate. Generally, however, dominant parr clearly suppressed the feeding activity of other parr. This was confirmed when several parr did not feed at all or fed infrequently as subordinates, but later dominated the feeding session after their superior had been removed from the aquarium.

Subordinates frequently made feeding-like movements toward food, indicating that their normal feeding activity was inhibited by the presence of the dominant.

FEEDING BEHAVIOR OF HATCHERY AND WILD SALMON AT THREE TEMPERATURES

Procedure

The temperatures of Cold Stream and the hatchery water supply were 1 and 3 C, respectively, so it was necessary to acclimate the fish for warmwater tests. This was done by holding them in heated indoor rearing troughs for 24 hr during each 3 C temperature change. All test groups were held the same length of time without feeding regardless of the time needed to bring them up to temperature. This resulted in a 1-week starvation period for all fish prior to testing.

Wild and hatchery salmon ranged in size from 76 to 88 mm, total length, and were matched to the nearest 2 mm. Four hatchery and four wild parr were placed simultaneously in separate compartments of the 181-liter aquarium and were held for 7 days at each temperature. For the first 2 days, the parr were left without feeding to become accustomed to the aquarium. Feedings and observations were made during the next 5 days, and then the fish were removed. Experimentally naive fish were used for each test group.

Each day at approximately 3:00 PM, parr in one compartment were fed 100 insects at a constant rate of one every 6 sec for a 10-min period. One man timed the event with a stop-watch and introduced the insects, while another man with a tape-recorder observed the fish and recorded the number of insects eaten by each fish. Immediately after the parr in one compartment were fed, parr in the opposite compartment were fed in the same manner. Hatchery and wild parr were fed in a random sequence throughout the experiment. The quantity of insects that four parr could consume in 10 min was predetermined. The fish were fed an excess of food, so the total amount of food consumed by the fish was therefore not dictated by the experimental situation. Each 10-min observation time was divided into two 5-min intervals, and insects eaten during these intervals were tallied separately so that effects of satiation could be noted.

Statistical analysis of data was accomplished using a 3×2^2 factorial experiment based on a random complete block design of temperature, type of fish, and time interval factors (Snedecor, 1956).

General Observations

As would be expected, the general activity of the parr increased with temperature. At 3 C feeding movements by the parr were leisurely. They generally rested on the substrate and returned to their stations after each feeding excursion. Feeding usually subsided after about 7 min, and the parr ignored the remainder of the food. Usually at least one of the parr occupied one of the boxes provided as shelter. Wild parr did not fight during the feeding session,

and no hierarchy was evident. Among hatchery parr, however, a definite hierarchy was set up, with a single dominant taking up a station at the center of the aquarium and the three subordinates lying at the periphery, displaying varying degrees of submissive coloration. The dominant attacked subordinates frequently during the feeding session.

At 12 C the parr were much more active than at the lower temperature. Wild parr either hovered over their stations or explored at mid-depth, feeding in rapid succession on insects as they entered the water. The parr fed rapidly throughout the 10-min period, although at a decreasing rate after about 8 min. Shelters were only occasionally occupied. Two- and sometimes three-way fights were observed among wild parr at this temperature, but no observable hierarchy was established. A strong hierarchy was again established among hatchery fish, with a dominant clearly suppressing the activities of subordinates.

At 19 C feeding activity was frenzied. The parr seldom contacted the bottom and spent much time exploring under the feeding tube, frequently nipping at the tube as they saw insects coming. The parr gorged themselves during the first 7 or 8 min, thereafter still responding to food but expelling a high percentage of the insects. Shelters were rarely used. Agonistic encounters were frequent, especially during the last half of the feeding session and immediately after feeding terminated. A weak hierarchy was established among wild parr, with one tending to dominate others but not noticeably suppressing their activities. As before, hatchery fish exhibited strong hierarchial behavior.

During this experiment it was noticed that at first hatchery parr had a tendency to reject insects more than wild parr. The parr would take insects into their mouths and expel them quickly, sometimes with a violent shaking of the head. This seemingly confused response to a food object appeared to be only momentary, for after a few insects were eaten the parr accepted them without hesitation.

Feeding Rates and Influence of Social Behavior

Daily total numbers of insects eaten by hatchery and wild parr over a 5-day period at each temperature are given in Fig. 1. Significantly fewer insects were eaten during the last 5-min interval than during the first 5-min interval at all temperatures ($F = 37.58$, 1 df), indicating that satiation was approached during the 10-min feeding period. There was a significant difference between feeding rates attained at the three temperatures ($F = 66.53$, 2 df). Feeding activity of the parr was lowest at the low temperature, and the smallest numbers of insects were consumed. The highest feeding rate recorded for both hatchery and wild parr was on the 1st day at the highest temperature. Thereafter, however, the most insects were consumed by fish held at the intermediate temperature. No attempt will be made to explain the large variation in feeding rates attained on different days at the different temperatures. The important results were that hatchery and wild parr differed significantly in total amount of food consumed ($F = 14.21$, 1 df). With the

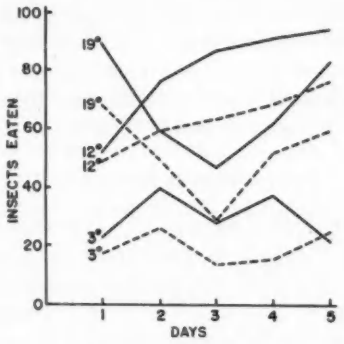

FIG. 1. Total numbers of insects eaten by four hatchery fish (broken lines) and four wild fish (solid lines) each day at the three temperatures tested.

one exception that occurred on the fifth day at 3 C, hatchery parr always consumed fewer insects than wild parr. There were no significant interactions between temperature, type of fish, and time interval ($F \leq 2.56$).

Although quantitative estimates of the level of agonistic activity were not recorded during the feeding sessions, it was evident that dominant hatchery parr were suppressing the activity of subordinates by repeated attacks, and that the dominants were getting a disproportionate share of the food. With few exceptions, the food consumption of a single dominant exceeded the combined food consumption of three subordinates (Table II). Wild parr shared their food more nearly equally, and, probably as a result, consumed a greater number of insects.

At the end of this experiment, dominant parr were successively removed from the aquarium in a manner similar to that described for the first exper-

TABLE II. Numbers of insects eaten by a single dominant and by three subordinate hatchery parr at three temperatures over a 5-day period.

Temp (C)	Fish type	Day 1	2	3	4	5
3	Dominant	4	10	1	12	1
	Subordinates	2	2	1	2	18
12	Dominant	42	50	42	39	46
	Subordinates	10	12	24	33	34
19	Dominant	40	28	15	12	16
	Subordinates	35	30	6	10	12

iment. The remaining fish were fed, and changes in food consumption by fish that were previously subordinate were noted. A fish that established dominance invariably consumed at least twice the amount of food it had eaten as a subordinate. This indicated that subordination in social relationships had an inhibitory effect on a parr's response to food in the aquaria.

DISCUSSION

Hatchery-reared landlocked salmon 10–12 months old were more socially aggressive than naturally reared salmon under our experimental conditions. This indicates that the normal development of social aggressiveness associated with territorial behavior in young salmon has been altered in the hatchery environment. From experiments conducted in an outdoor stream aquarium under more natural conditions, we have also obtained evidence that landlocked salmon exhibit social hierarchies superimposed on territorial behavior, and that the social interactions are more intense among hatchery-reared salmon (unpublished data). Male (MS, 1966) found that size is at least one of the determinants of social dominance in juvenile landlocked salmon: large fish tend to dominate smaller fish. Because size was equalized in our aquarium experiments, intrinsic factors must also be important in controlling the aggressiveness of salmon.

The most striking characteristic of the hatchery environment is the high density of fish maintained in rearing pools, which in Maine hatcheries can be as high as 145 fish/m^2 of substrate, probably many times greater than ever occurs in nature beyond the hatching and dispersion periods. Keenleyside and Yamamoto (1962) have raised the question as to what effect crowding might have on the development of territorial behavior of juvenile Atlantic salmon. They found that as the number of fish was increased to a high density in aquaria, agonistic encounters at first increased and then decreased. Agonistic activity was apparently inhibited at very high densities by the tendency for non-territory holding fish to form closely knit schools at the water surface, where they were seldom attacked by dominants.

Salmon parr have little choice but to aggregate or form schools in crowded rearing pools. It seems likely that mutual repulsion of individuals, which is normally expressed when young salmon are given enough space to establish minimum distances and territorial boundaries, must be suppressed. Other factors tend to discourage the formation of organized social relationships of hatchery fish. They are subjected to frequent disturbances attendant with the operation of the hatchery, and kept at high levels of excitation caused by visitors, mass feeding, cleaning of rearing pools, periodic sorting and weighing, chemical treatment for disease, and transfer from one area to another. If the full expression of aggressive behavior of young salmon is in part a learned response, as in many other animals (Scott, 1958), the conditioning effect of crowding may have adverse consequences. One could speculate that in a hatchery pool, fish are continually stimulated to fight, and that this continual stimulation may in time produce overly aggressive individuals. Studies now

in progress on the behavior of salmon in hatchery rearing pools indicate that there is indeed aggressive interaction of high intensity among hatchery fish held at high density.

Another possible reason for the apparent differences in the aggressiveness of hatchery-reared and wild salmon is that there may be a wide range in levels of innate aggressiveness in any population of a territorial species, and the hatchery environment has preserved the extremes of this variation. If this is true, the supposition that hatchery and wild salmon were genetically homogeneous would be false. That innate differences in aggressiveness between hatchery and wild salmon might exist was suggested in the first experiment when dominant hatchery fish exhibited a more variable nipping rate in agonistic encounters than wild fish. In another experiment at the Cobb Station, Male (MS, 1966) found evidence that hatchery-reared landlocked salmon are also more variable than wild salmon in their response to a fright stimulus under varying conditions of flow and light intensity. Hatchery salmon exhibited a strong fright reaction and shelter-orientation under shallow, fast-flow conditions and low light intensity, but they exhibited little or no response to a fright stimulus under deep, slow-flow conditions and high-light intensity. Wild salmon were consistently frightened by an overhead stimulus and exhibited strong shelter-orientation under all test conditions. Whether or not the greater variability of hatchery fish in this behavior was due to their inability to adjust to different environmental situations as the result of conditioning in the uniform hatchery environment, or whether they were displaying genetic variation in the expression of fright behavior, was not determined.

The lower feeding rates attained by hatchery fish in the second experiment probably do not reflect a deficiency in physiological adjustment to temperature, or a food preference different from that of wild salmon, but a behavioral maladjustment in social relations which interfered with feeding. That hatchery parr were at a disadvantage when fed natural food seems unlikely because, in the competitive situation of the first experiment, no differences in food consumption could be demonstrated between dominant hatchery and wild parr or between subordinate hatchery and wild parr. When separated in a second experiment, hatchery parr, because they were more inclined to fight and spend less time feeding consumed fewer insects than wild parr. The influence of differential effects of temperature can be ruled out because a disparity in food consumption occurred between hatchery and wild parr at 3 C in the second experiment, whereas in the first experiment, which was conducted at the same temperature, no disparity occurred when fish were compared on the basis of social status. However, it would have been a more valid procedure if single, isolated fish had been tested in feeding experiments to eliminate the influence of social interaction between individuals. Further experiments are planned, using isolated fish, to compare the preference of hatchery and wild salmon for various natural and unnatural foods.

Other laboratory artifacts could have biased results presented here and are being considered in future experiments. For example, crowding may affect

the behavior of wild and hatchery salmon differently. The normal tendency of wild salmon to fight and defend territory may have been suppressed at the high densities of fish existing in the aquaria, whereas the social behavior of hatchery fish may have been uninhibited in a situation more natural for them. It is also possible that wild salmon need more time to adjust to the aquarium environment than do hatchery salmon. If such was the case here, hatchery salmon may have dominated wild salmon simply because hatchery salmon overcame their initial fright reactions and became adapted to the new surroundings earlier than wild salmon.

In spite of the possibility of bias introduced by laboratory artifacts, the evidence presented here, plus evidence from other studies in our laboratories, suggests strongly that the aggressiveness of juvenile landlocked salmon reared in Maine hatcheries is unnaturally high. This behavior could contribute to mortality of hatchery fish stocked in natural streams through loss of feeding time, excessive expenditure of energy, and increased exposure to predators.

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Lactic Acidosis in Channel Catfish[1,2]

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ABSTRACT

Channel catfish (*Ictalurus punctatus*) were subjected to 5 or 10 min of anoxia (removed from water) to rapidly increase blood lactic acid concentration metabolically. Blood *p*H decreased as blood lactic acid concentration increased. Respiratory rate increased in fish with lowered blood *p*H and with increased blood lactic acid concentration during the 10 min after fish were returned to water after anoxia. Fish not treated had a mean blood *p*H of 7.8. Blood *p*H decreased in catfish subjected to anoxia 5, 10, and 15 min and decreased further when the fish were returned to water. Lowered blood *p*H was associated with loss of equilibrium and death in catfish subjected to transportation and handling.

INTRODUCTION

An association between accumulation of lactic acid in the tissues and mortality in fish subjected to hyperactivity has been reported (Buddenbrock, 1938; Secondat and Diaz, 1942; Black, 1957; Parker and Black, 1959; Parker et al., 1959; Miller and Miller, 1962; Caillouet, 1967) and discussed extensively (Black, 1958a, b; Black et al., 1959, 1961, 1962, 1966).

Jonas et al. (1962) injected lactic acid intravenously into rainbow trout (*Salmo gairdneri*) and sockeye salmon (*Oncorhynchus nerka*) and concluded that subsequent death was caused by the resulting decrease in blood *p*H. Hochachka (1963) showed a need for further study of buffering capacity of fish blood in relation to accumulation of lactic acid during muscular activity.

The present study was conducted to determine the association between blood *p*H and blood lactic acid concentration in channel catfish exposed to anoxia to metabolically increase lactic acid concentration in the tissues. Observations of blood *p*H in transported catfish were also made.

METHODS

For two experiments, immature channel catfish, weighing 41–114 g, were collected with hoop nets from the Des Moines River, Polk County, Iowa, and were transported in aerated tanks to the laboratory at Iowa State University on August 2, 1963. The fish were transferred to a 200-gal aerated tank in the laboratory and were allowed to recover. Fish that died or appeared

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injured were discarded. The tank was covered to limit disturbance of the fish.

In expt. 1 (Table I) on August 9, blood samples were taken from fish in five groups treated as follows: (1) control (no treatment); (2) subjected to anoxia (removed from water and held in a dip net) 5 min; (3) subjected to anoxia 10 min; (4) subjected to anoxia 5 min and returned to water for 10 min; and (5) subjected to anoxia 10 min and returned to water for 10 min. Fish were assigned randomly to treatments, two fish per treatment. Respiratory rate (number of opercular beats per minute) was measured in the four fish returned to water after anoxia at 0, 2, 4, 6, 8, and 10 min after return to water. Prior to anoxia, respiratory rate was measured in one of these fish. Water temperature was 20 C, and room temperature was 27 C.

In expt. 2 (Table II) on August 10, four control fish were sampled; then, seven others were simultaneously subjected to anoxia 10 min, were returned to water, and were sampled one after the other during a 63-min period. Respiratory rate was measured just before blood sampling in all fish returned to water after anoxia. Water temperature was 22 C, and room temperature was 26 C.

In both of these experiments, anoxia was used merely as a method of causing rapid accumulation of lactic acid in the tissues (see Leivestad et al., 1957; Scholander et al., 1962). At the time for sampling blood, each fish was stunned by a blow on the head, the operculum was lifted, and the hypodermic needle was inserted into the heart (see Perkins, 1957) while the fish was held out of water with a moist cloth. Blood was drawn into the chamber of a hypodermic style electrode assembly (No. 39022, Beckman Instruments, Inc., Fullerton, Calif.), and *p*H was read from a Beckman GS meter. The time between insertion of the needle and reading of *p*H averaged 1.3 min (range 0.8–2.7). A *p*H 7.0 buffer solution was used in calibration. Before receiving a blood sample, the sample chamber was flushed several times with distilled water and at least twice with 0.85% sodium chloride solution. Distilled water, sodium chloride solution, and buffer solution were kept at the same temperature as that of water in which the fish were held. Immediately after *p*H measurement, 1 ml or less of blood was extracted with a 2-ml Luer syringe into which aqueous ammonium heparinate (1000 USP heparin units per ml) had been drawn, and from which excess anticoagulant had been thoroughly ejected. The sample was immediately ejected into a polyethylene vial containing 9 ml of cold 10% trichloroacetic acid solution. Time for determining blood *p*H and for extracting blood for lactic acid analysis averaged 2.7 min (range 1.4–5.3). The mixture of blood and trichloroacetic acid was filtered within 15 min, and the filtrate, collected in a clean polyethylene vial, was stored at −25 C until analyzed for lactic acid by the colorimetric method of Barker and Summerson (Hawk et al., 1954).

In addition to the two experiments described, blood *p*H only was measured in mature and immature channel catfish, weighing 86–1574 g, that were transported on August 26 about 40 miles to the laboratory in non-aerated

containers. Before transportation, the fish had been held in aerated tanks for several weeks after being captured from various Iowa streams. Many of the fish had abrasions about the mouth, fins, and body from handling and transportation. In expt. 3, blood *p*H was measured in seven fish (one that appeared normal, five that lost equilibrium, and one that died) during 2 hr after transportation and transfer to the laboratory tank. Water temperature was 21 C. Nine more fish died within 1.5 hr after transportation, and two died in the next 2 days. The time required to determine blood *p*H averaged 0.8 min (range 0.6–1.0).

In expt. 4, the day after transportation, blood *p*H only was measured in fish that recovered in the laboratory tank; six were sampled without treatment (control), then 12 were simultaneously subjected to anoxia for 15 min and were returned to water to be sampled one after the other during a 50-min period. Water temperature was 16–18 C, and room temperature was 23 C.

RESULTS

Blood *p*H and Blood Lactic Acid

Tests of orthogonal comparisons among treatments in expt. 1 (Table I) indicated that mean blood *p*H was higher in control fish than in those subjected

TABLE I. Blood *p*H and blood lactic acid concentration (mg/100 ml) in immature channel catfish, two not treated (control) and eight subjected to anoxia in expt. 1 (water temperature, 20 C; room temperature, 27 C).

Treatment	*p*H	Lactic acid
Control	7.8	6.8
	7.8	8.5
Anoxia 5 min, not returned to water	7.5	16.6
	7.6	24.4
Anoxia 5 min, returned to water 10 min	7.2	25.6
	7.3	30.6
Anoxia 10 min, not returned to water	7.4	29.3
	7.5	22.8
Anoxia 10 min, returned to water 10 min	7.2	30.0
	7.2	61.5

Analysis of variance of blood pH and lactic acid values Source	df	Mean square	Mean square (with transformation)
Among treatments	4	0.118*[a]	0.157*
Control vs. subjected to anoxia	(1)	0.306*	0.516*
Anoxia 5 min vs. anoxia 10 min	(1)	0.011	0.043
Returned to water after anoxia vs. not returned to water after anoxia	(1)	0.151*	0.066
No meaningful comparison	(1)	0.002	0.003
Fish within treatments	5	0.003	0.015

[a]*Indicates significance at the 95% level of confidence ($P<0.05$).

to anoxia, and that it was higher in fish not returned to water after anoxia than in those returned to water after anoxia. There was no detectable difference in mean blood *p*H between fish subjected to anoxia for 5 min and those subjected to anoxia 10 min.

A similar analysis of variance was conducted on blood lactic acid data that were first transformed to common logarithms to assure homogeneity of variance. A logarithmic transformation was used because the coefficient of variation (standard deviation/mean) for different treatments in expt. 1 was relatively constant (see also Caillouet, 1967). Mean blood lactic acid concentration of control fish was lower than that of fish subjected to anoxia (Table I). No other orthogonal comparison among treatments was significant.

Thus, mean blood *p*H decreased and mean blood lactic acid concentration increased in fish subjected to anoxia. The decline in mean blood *p*H was greater in fish returned to water after anoxia than in those not returned to water after anoxia; however, mean blood lactic acid concentration was not significantly greater in fish returned to water than in those not returned to water after anoxia.

Again, in expt. 2 (Table II) mean blood *p*H decreased and mean blood lactic acid concentration increased in fish subjected to anoxia.

TABLE II. Blood *p*H and blood lactic acid concentration (mg/100 ml) in immature channel catfish, four not treated (control) and seven subjected to anoxia in expt. 2 (water temperature, 22 C; room temperature, 26 C).

Treatment	*p*H	Lactic acid
Control	7.8	4.4
	7.8	5.1
	7.8	7.1
	7.8	10.4
Anoxia 10 min, returned to water		
1 min	7.4	35.9
12 "	7.3	29.3
23 "	7.4	40.8
33 "	7.6	10.8
38 "	7.6	21.6
46 "	7.5	21.9
63 "	7.5	33.0

Analysis of variance of blood pH and lactic acid values Source	df	Mean square	Mean square (with transformation)
Among treatments	1	0.275*[a]	0.928*
Fish within treatments	9	0.008	0.035

[a]See footnote a, Table I.

The regression of blood *p*H on blood lactic acid concentration (expt. 1 and 2 combined) yielded the following equation:

$$\hat{Y} = 7.8 - 0.01X$$

where X = blood lactic acid concentration (mg/100 ml); Y = blood *p*H; $\hat{Y}$ = estimated blood *p*H. The mean square deviation from regression was 0.01 and the correlation coefficient was $r = -0.83$.

The mean square deviation from regression of blood *p*H on blood lactic acid concentration was significantly ($P < 0.05$) different in expt. 1 and 2; therefore, only approximate tests of differences between slopes and elevations of the two regression lines could be made. Analysis of covariance detected no significant differences in slope or elevation of the two regression lines.

There was a significant linear increase in respiratory rate with time during the 10 min that fish were left in water after anoxia in expt. 1 (Table III). Fish subjected to anoxia 10 min exhibited a higher mean respiratory rate

TABLE III. Respiratory rate (number of opercular beats per minute) in four immature channel catfish subjected to anoxia and returned to water for 10 min in expt. 1.

Treatment	Time after anoxia (*min*)						Mean
	0	2	4	6	8	10	
Anoxia 5 min	92	89	96	93	93	85	91
	98[a]	103	102	105	108	108	104
Anoxia 10 min	102	100	101	102	102	103	102
	95	98	102	102	106	111	102
Mean	97	98	100	100	102	102	100

[a]The respiratory rate of this fish was 95 beats per min before anoxia. Its blood *p*H was 7.3 after the fish had been in water 10 min after anoxia; blood *p*H was 7.2 in the other three fish 10 min after anoxia.

Analysis of variance respiratory rate values

Source	df	Mean square
Time	5	20.20
Linear regression	(1)	89.16*[a]
Deviations	(4)	2.96
Fish[b]	3	198.67*
Anoxia 5 min vs. anoxia 10 min	(1)	112.67*
No meaningful comparisons	(2)	241.66*
Error (time x fish)	15	14.20

[a]See footnote a, Table I.
[b]Orthogonal comparisons among fish.

than that of fish subjected to anoxia 5 min. Mean respiratory rate of fish returned to water after anoxia in expt. 2 was 126 beats per minute (range 103–152) which was higher than that, 100 beats per minute (range 85–111), for fish treated similarly in expt. 1 (Table III). The higher respiratory rate may have resulted from higher water temperature in expt. 2, since mean blood *p*H and mean blood lactic acid concentration of fish subjected to anoxia and returned to water did not differ significantly in expt. 1 and 2.

The mean blood *p*H of all control fish was 7.8 (Table IV). This included fish that had recovered from transportation in expt. 4. During the first 30

TABLE IV. Blood *p*H in channel catfish not treated (control), subjected to anoxia, or transported.

Expt. No.	Treatment	No. of fish	Blood *p*H	
			Mean	Range
1,2,4	Control	12	7.8	7.7–7.8
	Anoxia 5 min			
1	Not returned to water	2	7.6	7.5–7.6
1	Returned to water 10 min	2	7.2	7.2–7.3
	Anoxia 10 min			
1	Not returned to water	2	7.4	7.4–7.5
2	Returned to water 1 min	1	7.4	–
1	Returned to water 10 min	2	7.2	–
2	Returned to water 12–23 min	2	7.4	7.3–7.4
2	Returned to water 33–63 min	4	7.6	7.5–7.6
	Anoxia 15 min			
4	Returned to water 6–35 min	9	7.2	7.1–7.2
4	Returned to water 38–50 min	3	7.3	7.2–7.3
	After transportation			
3	Appeared normal	1	7.1	–
3	Lost equilibrium	5	6.8	6.7–6.9
3	Dead	1	6.4	–

min or so after fish exposed to anoxia were returned to water, blood *p*H was near 7.2, but the rate of recovery seemed less rapid in fish exposed to anoxia 15 min than in those exposed to anoxia for shorter periods. Fish exposed to anoxia and returned to water did not approach blood *p*H of control fish within the sampling period of 1 hr or so.

Fish sampled within 2 hr after transportation and transfer to the laboratory tank (expt. 3, Table IV) had the lowest blood *p*H encountered during this investigation. Of these, the fish that appeared normal exhibited the highest blood *p*H, those that lost equilibrium had an intermediate mean blood *p*H, and the dead fish had the lowest blood *p*H, 6.4.

DISCUSSION

In this study, blood *p*H of control channel catfish was higher than that reported by Haws and Goodnight (1962); viz., 7.49–7.65 (10 fish). Auvergnat and Secondat (1942) observed that blood *p*H was lower in carp, *Cyprinus carpio*, subjected to 15 min of forced exercise than in "resting" fish. Increased acidity of the blood was accompanied by decreased plasma carbon dioxide. They attributed the decrease in *p*H and carbon dioxide to an increase in lactic acid concentration of the blood, because Secondat and Diaz (1942) had shown that blood lactic acid concentration increased in tench, *Tinca tinca*, exercised for 15 min. Black et al. (1959) observed that blood *p*H and blood carbon dioxide levels of rainbow trout decreased as blood lactic acid concentration increased during and after 15 min of exercise. They, too, concluded that carbon dioxide concentration was lowered by the increase in lactic acid concentration of the blood. Similar conclusions were reached by Black et al. (1966). Not only did neutralization of lactic acid occur at the expense of bicarbonate (Secondat and Diaz, 1942), but this buffering action seemed insufficient to prevent decrease in blood *p*H during exercise. Jonas et al. (1962) lowered blood *p*H in rainbow trout and sockeye salmon by intravenous injection of lactic acid. The decrease in blood *p*H with increase in blood lactic acid concentration in channel catfish is interpreted as a failure of the buffering systems of the blood to neutralize the lactic acid.

Lactic acidosis, an accumulation of hydrogen ions and a more acid reaction of the body fluids, caused almost entirely by lactic acid, has resulted in death in humans (Huckabee, 1961a, b). A major effect of acidosis is depression of the central nervous system (Guyton, 1961). If severe enough, acidosis can cause disorientation and coma in humans. Loss of equilibrium in transported channel catfish suggests depression of the central nervous system by increased acidity of the tissues. Increased ventilation, indicated by increase in respiratory rate, is another symptom of acidosis that was exhibited by channel catfish with lowered blood *p*H and increased blood lactic acid concentration.

Buddenbrock (1938) suggested that deformation of erythrocytes and reduction of oxygen capacity of the blood, caused by accumulation of lactic acid, might lead to tissue hypoxia in fish and that hypoxia would further enhance lactic acid production. Root (1931), Black and Irving (1938), and Root and Irving (1943) demonstrated that oxygen capacity of fish blood decreased with increase in lactic acid concentration. Sedimentation of erythrocytes within smaller vessels (Black, 1956) and reduced circulatory efficiency (Hayashi, cited by Black et al., 1962) in fish could reduce oxygen transport to the tissues and cause hypoxia. Black et al. (1962, 1966) reported that tissue hypoxia occurred after strenuous muscular exercise in rainbow trout. The blood of channel catfish that lost equilibrium or died after transportation, was darker than normal, and this suggested that it was poorly oxygenated.

When blood *p*H fell below 6.8–6.9 after intravenous injection of lactic acid into two salmonids, death usually occurred (Jonas et al., 1962). Blood

*p*H of 6.7–6.9 was observed in channel catfish that lost equilibrium after transportation, and a dead catfish had a blood *p*H of 6.4.

It has been demonstrated that anoxia, hyperactivity, and capture can cause accumulation of lactic acid in the blood of channel catfish (Caillouet, 1967). In the present study, it was shown that blood *p*H decreased as blood lactic acid concentration increased in channel catfish exposed to anoxia, that respiratory rate increased in these fish, and that loss of equilibrium and death were associated with low blood *p*H in transported fish. It is conculded that such symptoms indicate lactic acidosis in channel catfish.

Blood *p*H requires less time and is easier to determine electrometrically than the colorimetric determination of blood lactic acid concentration. It is suggested that blood *p*H may be a more practical and reliable indicator of condition in fish subjected to hyperactivity than is concentration of lactic acid in the blood.

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Turnover and Urinary Excretion of Free and Acetylated M.S. 222 by Rainbow Trout, *Salmo gairdneri*[1]

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ABSTRACT

Rainbow trout (*Salmo gairdneri*) anesthetized in 100 mg/liter of M.S. 222 at 12 C excreted the drug in free and acetylated forms via the urine during a 24-hr recovery period in freshwater. Of the M.S. 222 excreted, 77–96% was acetylated. Blood levels of free drug in anesthetized trout approximated 75% of the anesthetic concentration, but the amount of acetylated M.S. 222 was relatively insignificant. The blood and urine were cleared of the two fractions of M.S. 222 in 8 and 24 hr respectively. Low levels of aromatic amines of natural origin occurred in blood and urine and were subtracted from measurements of M.S. 222. Intraperitoneal injections of 10–100 mg/kg of M.S. 222 did not induce anesthesia; however, the 24-hr pattern of drug excretion was similar to that observed after anesthesia by immersion. Only 15–21% of the injected dose was found in the urine, suggesting a second route of drug elimination.

INTRODUCTION

M.S. 222 (tricaine methanesulfonate, Sandoz), a primary aromatic amine, is used commonly to anesthetize fish for marking, transport, spawn-taking, surgery, urinary catheterization, and blood sampling. Residues of this drug occur in various tissues of anesthetized trout and catfish, but decline to background levels within 9–24 hr after fish are placed into freshwater (Walker and Schoettger, 1967b; Schoettger et al., 1967). Other than the fact that the residues do disappear, little is known about the metabolic fate of M.S. 222. The above authors made some measurements of acetylated M.S. 222 in skeletal muscle of trout and catfish, but did not conclude definitely that the mechanism of drug elimination was acetylation and/or excretion. Oser (1965) states that exogenous amines are acetylated and excreted in the urine. Our study was undertaken to determine the role of the rainbow trout kidney in excreting free and acetylated M.S. 222.

MATERIALS AND METHODS

The rainbow trout used in this study were the Manchester strain, obtained from the National Fish Hatchery, Manchester, Iowa. They ranged in weight from 305 to 660 g.

Urine to be analyzed for M.S. 222 was collected by catheterizing the fish and placing them in plexiglass chambers containing flowing well water at 12 C (Fig. 1).

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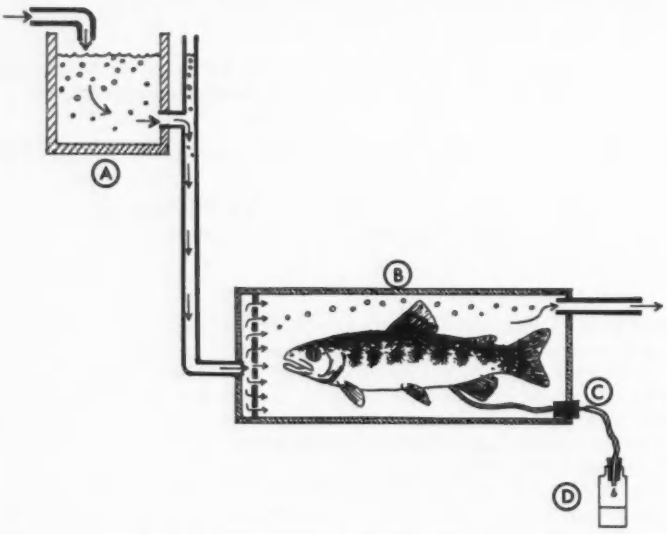

FIG. 1. Plexiglass chamber used in urine collections. A, headbox; B, plexiglass chamber 18 × 3 × 5 inches, made from ¼-inch clear plexiglass; C, No. 0 rubber stopper with 21-gauge hypodermic needle; D, polyethylene bottle with slotted rubber stopper.

Two types of experiments were conducted. In the first, we anesthetized two groups of trout in 100 mg/liter M.S. 222 for 2.0–2.5 min. Urine was collected at timed intervals from one group, and blood from the other. The latter group contained eight subgroups of four fish each. The subgroups were sampled at intervals identical with those used for urine collection (Table I). They were then reanesthetized with 5 ml/liter methylpentynol and blood was collected with heparinized needle and syringe from vessels in the caudal peduncle just posterior to the anal fin.

In the second type, we wished to determine the quantity of background aromatic amines in the urine of anesthetized fish, and the relative amount of injected M.S. 222 which is excreted via the kidney. One group of fish was anesthetized in 3 ml/liter of methylpentynol for 5 min and their urine analyzed for free and acetylated aromatic amines at intervals over 48 hr. A second group was similarly anesthetized and injected intraperitoneally with M.S. 222 dissolved in 0.85% NaCl. The dose of 10 mg/kg body weight was administered immediately behind the pelvic fins. We had determined previously that this dosage of M.S. 222 had no observable effect on the behavior of the fish. Urine samples from these fish were analyzed at intervals over 24 hr.

The catheterization procedure is as follows: (1) after anesthetization the fish are blotted dry in an absorbent towel, weighed, and placed on their backs

TABLE I. Concentrations of free and acetylated M.S. 222 in urine and whole blood of rainbow trout after anesthesia in a solution containing 100 mg/liter of drug at 12 C.

Time post-anesthesia (*hr*)	Urine		Whole blood	
	Free M.S. 222 (*mg/liter*)	Acetylated M.S. 222 (*mg/liter*)	Free M.S. 222 (*mg/liter*)	Acetylated M.S. 222 (*mg/liter*)
0	No measurement	No measurement	73.6[a] (4)[b] 39.4–95.0[c]	1.8[a] (4) 0.0–6.3
0.5	Do	Do	6.7 (4) 5.0–8.4	1.0 (4) 0.1–1.3
1	Do	Do	4.6 (4) 3.4–5.6	0.3 (4) 0.0–0.5
2	9.2[d] (7)[b] 6.5–14.1[c]	26.4[d] (7) 13.4–44.6	2.2 (4) 1.0–3.0	0.8 (4) 0.0–1.5
4	4.8 (7) 2.8–6.6	35.5 (7) 15.6–61.6	0.6 (4) 0.0–2.0	0.1 (4) 0.0–0.3
6	3.1 (6) 2.4–4.2	21.9 (6) 6.4–50.4	0.8 (4) 0.0–1.6	0.4 (4) 0.0–1.1
8	1.8 (5) 1.4–2.4	10.9 (5) 3.1–18.1	0.0 (4)	0.0 (4)
12	0.6 (5) 0.0–0.9	4.5 (5) 0.9–8.3	0.0 (4)	0.0 (4)
24	0.2 (6) 0.0–0.7	0.3 (6) 0.0–1.5	–	–

[a]Mean concentrations corrected for free or acetylated aromatic amines of natural origin in blood of control fish.
[b]Number of fish.
[c]Range.
[d]Mean concentrations corrected for free or acetylated aromatic amines of natural origin in urine (Table III).

in a towel-covered V-trough; (2) a catheter of No. 5 polyvinyl tubing[2] is inserted into the urinary bladder and sutured in place; (3) the catheter is secured by a second suture to the anal fin; (4) fish are then placed into the plexiglass chamber (Fig. 1), the free end of the catheter is attached to the catheter outlet, and the water is turned on. With practice, the entire operation can be completed in about 8 min. We noted that the flow of urine was intermittent. To obtain sufficient urine for analyses of both free and acetylated M.S. 222 at selected time intervals, it was allowed to accumulate in the collecting vials for a minimum of 2 hr.

[2]Intravenous tubing, surgical grade vinyl. Bel-Art Products, Pequannock, N.J.

Free and acetylated M.S. 222, and background aromatic amines in whole blood and urine, were determined by the Bratton = Marshall method as modified by Walker and Schoettger (1967a).

Free and acetylated aromatic amines of natural origin occurred in the urine of methylpentynol-anesthetized trout which had no previous exposure to M.S. 222 (Table III). Since these amines react in the analytical method for M.S. 222, we considered the concentrations as estimates of background interference. The values were used to correct measurements of M.S. 222 (Tables I, II) in trout urine. The mean levels of free, natural amines ranged from 0.6 to 1.5 mg/liter and do not appear to be associated with post-anesthesia time. However, the average levels of acetylated, natural amines declined from about 2.0 mg/liter to 0 over 48 hr and seem associated with post-anesthesia time. Conceivably, anesthesia or handling may influence the level of natural, acetylated amines in trout urine.

TABLE II. Concentrations of free and acetylated M.S. 222 in the urine of rainbow trout after anesthesia in methylpentynol and injection of 10 mg/kg of M.S. 222.

Time post-anesthesia (*hr*)	No. of fish	Free M.S. 222 (*mg/liter*)	Acetylated M.S. 222 (*mg/liter*)
4	5	4.2[a] 2.0–11.5[b]	40.5[a] 13.4–54.4
8	5	3.2 1.4–6.4	34.2 19.1–50.1
12	5	1.2 0.0–2.5	11.5 2.3–23.9
24	5	0.5 0.0–1.1	3.1 0.5–6.5

[a]Mean concentration corrected for free or acetylated aromatic amines of natural origin (Table III).
[b]Range.

The mean background levels of free and acetylated, aromatic amines of natural origin in whole blood were 1.6 (0.8–2.4) mg/liter and 0.3 (0.0–0.8) mg/liter respectively. The determinations were made on blood samples taken from trout immediately after methylpentynol anesthesia.

RESULTS

Analyses of the urine from rainbow trout which were anesthetized in M.S. 222 show that both free and acetylated forms of the drug are excreted

TABLE III. Concentrations of free and acetylated aromatic amines occurring naturally in the urine of rainbow trout after anesthesia in methylpentynol at 12 C.

Time post-anesthesia (*hr*)	No. of fish	Urine	
		Free aromatic amines (*mg/liter*)	Acetylated aromatic amines (*mg/liter*)
2	3	1.5[a] 1.0–1.0[b]	1.8 0.8–2.6
4	4	1.0 0.8–1.6	2.2 1.4–3.8
6	4	0.6 0.4–0.8	1.2 0.6–2.6
8	4	0.6 0.4–0.8	0.5 0.0–1.6
12	4	1.1 0.8–1.4	0.1 0.0–0.6
24	4	0.9 0.8–1.0	0.1 0.0–0.2
48	4	0.8 0.6–1.0	0.0

[a]Mean.
[b]Range.

renally (Table I). The free fraction declined from an average of 9.2 mg/liter at 2 hr after anesthesia to 0.2 mg/liter at 24 hr. On the other hand, the mean concentration of acetylated M.S. 222 increased from 26.4 mg/liter at 2 hr post-anesthesia to 35.5 mg/liter at 4 hr, and then declined to 0.3 mg/liter at 24 hr. Also, M.S. 222 injected intraperitoneally was excreted in both the free and the acetylated forms (Table II). In both cases, acetylated M.S. 222 accounted for 77–96% of the M.S. 222 excreted renally.

The preponderance of acetylated M.S. 222 found in urine was not paralleled by equally large amounts of acetylated drug in blood. The free fraction was the major form found in the blood after M.S. 222 anesthesia (Table I). At 0-hr withdrawal the average level of free M.S. 222 was 73.6 mg/liter whereas the acetylated fraction was only 1.8 mg/liter. Both fractions were reduced to background levels during 8 hr of recovery.

Although M.S. 222 was excreted renally, 79–85% of the injected dose was excreted extrarenally (Table IV). Preliminary investigations on the narcotic, anesthetic effects of injected M.S. 222 showed that it is ineffective in rainbow trout at doses ranging from 10 to 100 mg/kg. This suggests that the rate of excretion via the extrarenal route, presumably the gills, is of a

TABLE IV. Total free and acetylated M.S. 222 excreted in urine of rainbow trout during 24 hr after intraperitoneal injections of 10 mg/kg of M.S. 222.

	Fish No.				
	1	2	3	4	5
Body weight (*g*)	350	480	480	515	445
Urine volume (*ml*)	35.8	42.1	38.9	41.9	59.8
Free M.S. 222 (*μg*)	58.7	84.7	189.7	32.1	43.6
Acetylated M.S. 222 (*μg*)	675.2	843.2	645.7	732.6	620.7
% dose excreted via kidney	21.0	19.3	17.4	15.0	15.1

magnitude sufficient to keep the blood level of free M.S. 222 below the effective concentration.

DISCUSSION

The rate of recovery of fish from M.S. 222 anesthesia probably depends on the rate of metabolism and/or excretion of the anesthetic. Schoettger and Julin (1967) indicate that 8.5–13.4 min are required to bring rainbow trout to the level of medullary collapse in 100 mg/liter of M.S. 222 at 12 C, with recovery in freshwater requiring 4.3–9.2 min. Our data, and those of Walker and Schoettger (1967b), indicate that this rapid recovery is associated with a decline in the levels of free M.S. 222 in the blood. The quantity of acetylated M.S. 222 in the blood remains at a low level during the recovery period. The blood is cleared of M.S. 222 during 8 hr of recovery.

Walker and Schoettger (1967b) reported that the concentrations of M.S. 222 in various tissues of rainbow trout declined to background levels after 9–24 hr of withdrawal. The results of our study show that M.S. 222 is cleared from the urine of rainbow trout within a similar period.

Acetylated M.S. 222 was found in much higher concentrations in the urine than in the blood of rainbow trout. This urine-to-blood ratio of acetylated M.S. 222 suggests either a concentration of the drug metabolite by the kidney, or that M.S. 222 is acetylated in the kidney and secreted into the urine. Gardiner (1961) states that acetylation of aromatic amines occurs in the liver of mammals and birds, but no mention is made of the kidney. Further studies should provide an answer to this interesting question.

Initially, the finding that M.S. 222 in the urine of rainbow trout was primarily in the acetylated form suggested that the renal route was paramount in the elimination of the drug. However, the fact that we observed low circulating levels of acetylated M.S. 222, and high levels of free M.S. 222 which are rapidly dissipated, suggests that most of the anesthetic was excreted in the free form. Furthermore, injection studies showed that only 15–21% of the injected dose was excreted renally. Therefore, during the recovery of

M.S. 222-anesthetized fish, much of the drug in the free form is excreted extrarenally, presumably across the gills. Acetylation and urinary excretion probably serve as a secondary route of drug elimination.

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Distributions of Juvenile Steelhead and Cutthroat Trout (*Salmo gairdneri* and *S. clarki clarki*) Within Streams in Southwestern British Columbia[1]

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ABSTRACT

Trout were collected and identified from 66 streams or stream systems of different size and gradient. Total dissolved solids (T.D.S.) and *p*H were determined on most streams. Size and profile of streams to a large degree determined the species of trout present. Large streams, with drainage area over 130 km^2, were predominantly occupied by steelhead. Small streams, drainage area under 13 km^2, were predominantly occupied by cutthroat. Streams less than 120 km^2 in drainage area with steep gradients, and emptying directly into the sea, usually supported steelhead, as did large rivers. Those which dropped steeply and then levelled and ran through several miles of sloughs usually supported cutthroat. Where both species occurred, cutthroat were most often predominant in the small tributaries and headwaters, and steelhead in the lower reaches of the main stream. Stream *p*H's were usually lower in winter than in summer, but had no obvious effect on trout distribution. Many cutthroat streams had high T.D.S. readings in the lower reaches in summer and low T.D.S. readings in these areas in winter. Otherwise there were no marked differences between steelhead and cutthroat streams in terms of T.D.S.

INTRODUCTION

The distributions of different species of fish within stream systems have been related to a number of physiographic factors, including temperature, *p*H, gradient, and size (Shelford, 1911; Thompson and Hunt, 1930; Trautman, 1942; Burton and Odum, 1945; Starmack, 1956; and Huet, 1959, 1962). Previous studies on distributions of salmonids in southwestern British Columbia (Hartman, 1965) showed that juvenile steelhead trout usually existed in close association with underyearling coho salmon along the lengths of streams. Steelhead and cutthroat trout juveniles, on the other hand, appeared to be segregated into streams of different sizes or into different areas of the same stream system.

In the present study a large number of collections of trout were made in a series of streams of different sizes and gradients in an effort to describe more fully the differences in distribution of young steelhead and cutthroat. The field work for the investigation was carried out between 1960 and 1966. Most of the data were gathered in 1964 and 1965. Stream profiles were determined and measurements of *p*H, total dissolved solids, temperature, and stream

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discharge were taken to determine if any of these factors bore an obvious relationship to the distribution of one species of trout or the other. The work is not intended to describe completely the distributional differences between the two species but is presented rather as a preliminary to a better understanding of their ecological relationships.

STUDY AREA, MATERIALS, AND METHODS

The 66 streams sampled lie in the following regions of southwestern British Columbia: the east coast of Vancouver Island, 16 streams (Fig. 1);

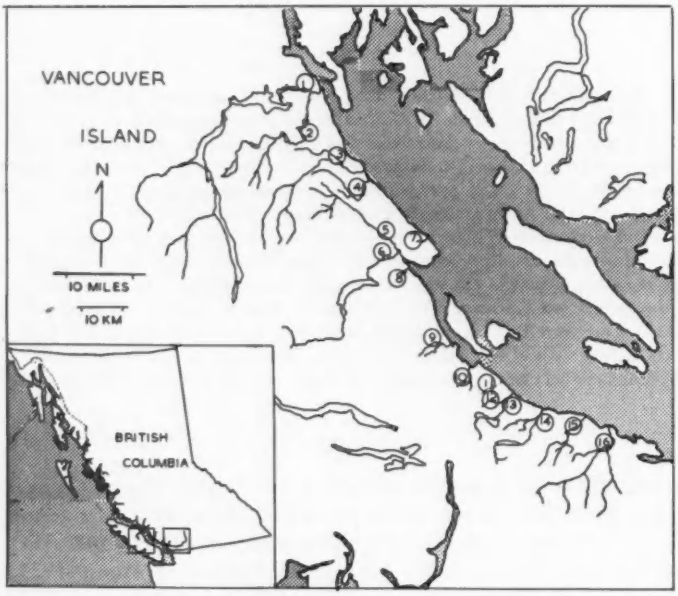

FIG. 1. Locations of streams sampled on the east side of Vancouver Island. Names of streams given in Table I (see text). Inset map shows locations of the two major sampling areas.

the Squamish area, 7 streams; and the lower Fraser Valley, 43 streams (Fig. 2). Names corresponding to stream numbers on Fig. 1 and 2, and approximate drainage areas, are given in Table I. The smaller streams are characterized by high winter and low summer discharge. The larger rivers normally have winter and summer run-off maxima, corresponding with heavy rainfall and snowmelt farther inland respectively.

Wherever access permitted, collections were made at a series of sites along the full length of each stream. Some inaccessible streams were sampled only at one location.

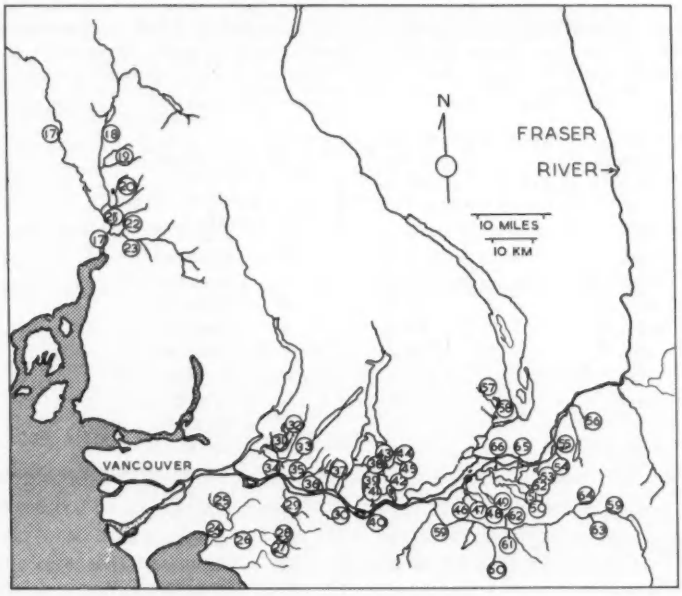

FIG. 2. Locations of streams sampled in the lower Fraser Valley and Squamish area. Names of streams given in Table I (see text).

Most collections were made with 3 × 2 m and 9 × 2 m seines with mesh size approximately 7 mm stretched mesh.

A 440 v, d-c fish shocker, Smith Root Laboratories Type IV, was used to make a limited number of collections. In situations where water depth or bottom topography prevented seining, fish were angled with No. 14 or No. 16 hooks and bait.

In many locations where streams were turbid or very turbulent, explosives were detonated in the water a few meters upstream from a positioned seine net, as described by Hartman (1965).

Measurements of *p*H were taken with a portable *p*H meter, Beckman N or E.I.L., 30C, within 1 min of the time the water sample was drawn from the stream. Total dissolved solids (T.D.S.) of stream water samples were determined in the laboratory with a conductivity bridge, Industrial Instrument R.C.7. Stream discharge data and some drainage areas were obtained from publications of Canada Department of Northern Affairs and National Resources (1960–1963). Stream discharges were estimated at collection sites by visual observations of cross section and velocity, where possible. Data on water chemistry, stream discharge, and drainage area are on file with the Fish and Wildlife Branch.

Trout were identified using six characters which were diagnostic on hatchery-reared fish of known parenthood (Hartman, MS, 1956). The characters were hyoid teeth, hyoid red pigmentation, mid-dorsal parr marks,

TABLE I. Names and drainage areas of streams shown in Fig. 1 and 2. Drainage areas with an asterisk (*) are from Water Resources Branch Papers (Canada Department of Northern Affairs and National Resources, 1960–1963). Other areas were calculated with a planimeter.

No.	Stream	Drainage area (km^2)
Fig. 1		
1	Campbell R.	1461.0*
2	Quinsam R.	277.1*
3	Oyster R.	181.3*
4	Black Cr.	65.1
5	Tsolum R.	251.5*
6	Puntledge R.	518.0*
7	Little R.	4.2
8	Millard Cr.	3.6
9	Cougar Cr.	24.0
10	Chef Cr.	21.0
11	Nile Cr.	17.9*
12	Hunts Cr.	16.4
13	Big Qualicum R.	147.6*
14	Little Qualicum R.	246.1*
15	French Cr.	73.3
16	Englishman R.	287.5*
Fig. 2		
17	Squamish R.	1981.3*
18	Cheakamus R.	813.3*
19	Culliton Cr.	66.7
20	Brohm Cr.	24.5
21	Schoonover Cr.	6.2
22	Mashiter Cr.	46.0
23	Mamquam R.	384.0
24	Serpentine R.	105.0
25	Mahood Cr.	19.7*
26	Nicomekl R.	99.5
27	Anderson Cr.	29.0
28	Murray Cr.	33.0
29	Salmon R.	83.0*
30	Nathan Cr.	35.0
31	Blaney Cr.	18.9
32	Blaney Cr. (east trib.)	2.0
33	S. Alouette R.	205.0*
34	S. Alouette R. trib.	2.0
Fig. 2 (*con't*)		
35	Kanaka Cr.	55.0
36	York Cr.	5.3
37	Whonock Cr.	20.0
38	Steelhead Cr.	14.6
39	Silverdale Cr.	21.2
40	St. Mary's Cr.	2.5
41	Draper Cr.	5.6
42	Hatzic Sl.	55.0
43	Legace Cr.	5.2
44	Allan L. outlet Cr.	9.4
45	Pattison Cr.	7.3
46	Atchelitz Cr.	7.4
47	Luckakuck Cr.	4.8
48	Chilliwack Cr.	15.2
49	Semmihault Cr.	8.3
50	Elk Cr.	16.0
51	Ford Cr.	8.5
52	Nevin and Dunnville cr.	18.0
53	Bridal Falls Cr.	13.0
54	Fraser R. trib. ¼ mile west of Jones Cr.	7.0
55	Fraser R. trib. ¼ mile east of Jones Cr.	1.1
56	Lorenzetta Cr.	35.0
57	Sakwi Cr.	18.6
58	Weaver Cr.	48.2
59	Chilliwack R.	1250.0*
60	Liumchen Cr.	52.0
61	Chilliwack R. trib. ¼ mile west of Tamihi Cr.	6.0
62	Ryder Cr.	7.3
63	Chilliwack R. trib. 5 miles east of Foley Cr.	2.4
64	Foley Cr.	80.0
65	Hope Sl.	85.7
66	Camp Sl.	13.6

maxillary length, and dorsal fin shape and color. Many trout under 1-year old, and some of those 1–2 years old, could not be classified with certainty, even though six characters were used. These fish are included in the results because of the possibility that they represent hybridization in some situations, particularly where both species are known to occur.

Stream profiles were calculated from topographic maps (British Columbia Surveys and Mapping Branch) with scales of 1:50,000 and 1:126,720. In cases where maps showed different elevations at a known point, the data were taken from the larger scale map.

Stream profiles shown (Fig. 3–7) begin at the ocean or where the stream in question enters a substantially larger tributary. Where a system consisting of two or more tributaries is shown, tributaries are placed in the horizontal scale to indicate where they enter the main stem of the system (see Salmon River, Fig. 7). If only a segment of a stream is shown (as is the case with the longer streams) the upper end of the profile is delimited with two diagonal lines.

RESULTS

Stream Size and Trout Distribution

The principal differences in distribution between steelhead and cutthroat juveniles related to stream size and profile. Steelhead trout appear to be adapted to large rivers or swift tributaries. In 34 of 40 collection sites on the mainstems of 13 large streams, drainage areas greater than 130 km² (50 miles²), the identifiable trout were steelhead (Fig. 3). Cutthroat trout made up a minor part of the six other collections.

Cutthroat trout were found in the very small streams or in headwaters. In the 21 small streams with drainage areas up to 13 km² (5 miles²), all identifiable fish were cutthroat in 24 out of 33 collections and steelhead in 5 out of 33 collections (Table I, Fig. 4–7). Both species occurred in 3 collections.

Table II, showing the numbers of streams in various drainage area categories in which one species of trout or the other or both occur, indicates that cutthroat trout are predominantly a small stream form. In the four large streams where they occurred, cutthroat made up a minor portion of the trout fauna. Steelhead occurred frequently over the range of stream sizes investigated.

Effect of Stream Profiles

If all streams with drainage areas under 120 km² (46 miles²) are considered it becomes evident that stream profile as well as size affects the trout species composition. The nine streams of this size in which all the identifiable trout were steelhead drop steeply into a large river or the ocean (Fig. 4). In those streams in which the identifiable trout were cutthroat the gradient is low to moderate (Fig. 5) or is near level and then increases sharply (Fig. 6). Eleven of these streams drain into large slough systems.

Spawning cutthroat trout enter Hope and Camp sloughs but young cutthroats were not obtained in these bodies of water. It is presumed that cutthroat spawners move through the sloughs and spawn in the steep tributaries. Figure 6 shows that cutthroat occur in streams with steep profiles. In all cases except Chef Creek, however, these streams flatten and run through an

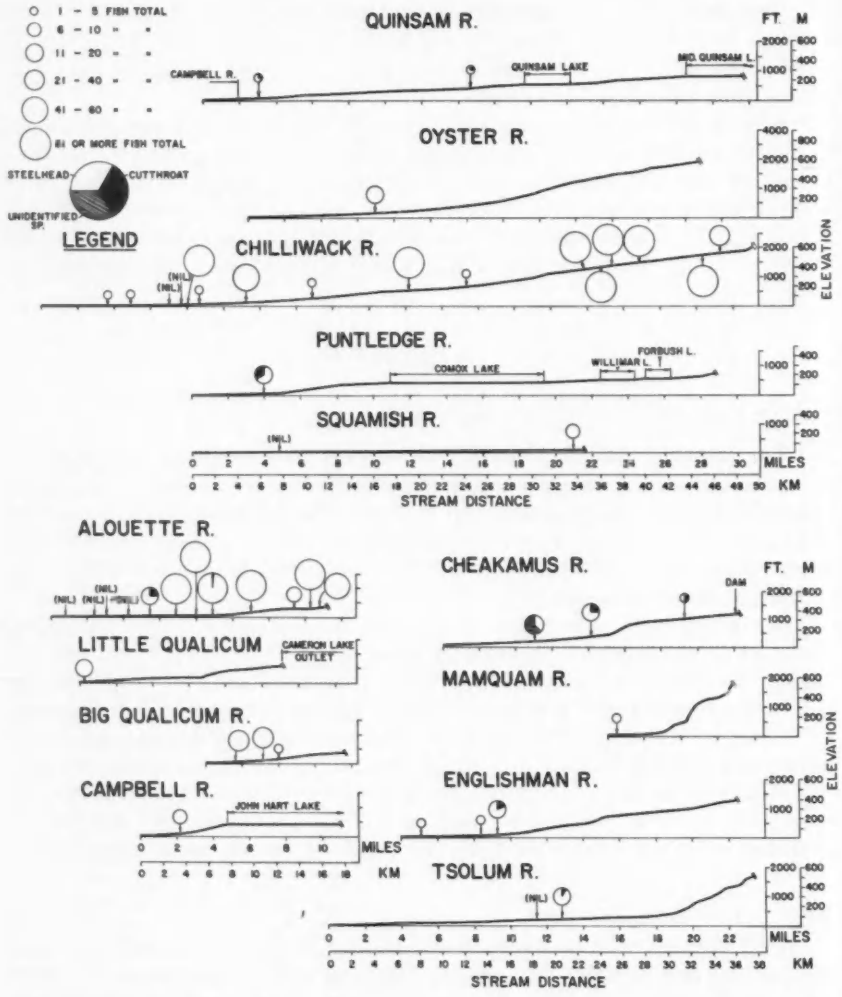

FIG. 3. Profiles of streams with drainage areas greater than 130 km^2, showing locations of sampling stations and sizes and compositions of trout collections. Legend showing sample sizes and composition applies to Fig. 3–7, all of which have the same scale. See text for further explanation of Fig. 3–7.

extensive area of meadowland or slough. The profiles of medium and small streams occupied by cutthroat trout are usually different than those occupied by steelhead trout. However, within either type of stream, cutthroat and steelhead trout were each generally found in riffle-pool complexes or in rocky

TABLE II. Numbers of streams of different drainage areas in which the trout sampled were cutthroat only, cutthroat and steelhead, or steelhead only.

Stream drainage area (km^2)	No. of streams with: Cutthroat	Cutthroat & steelhead	Steelhead
0–40	23	11	6
41–80	0	4	4
81–120	0	3	0
121–160	0	0	1
161–200	0	0	1
>200	0	4[a]	7

[a]Cutthroat comprised about 3 and 12% of the trout in two of the four streams and 25 and 29% in the other two.

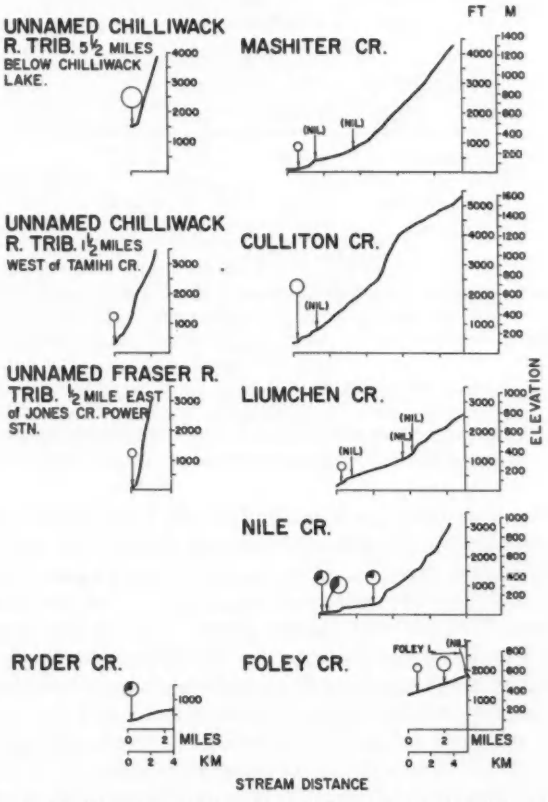

FIG. 4. Profiles of streams with drainage areas under 120 km^2 in in which all identifiable trout were steelhead. Sampling stations, sample size, and composition are indicated. See Fig. 3 for legend.

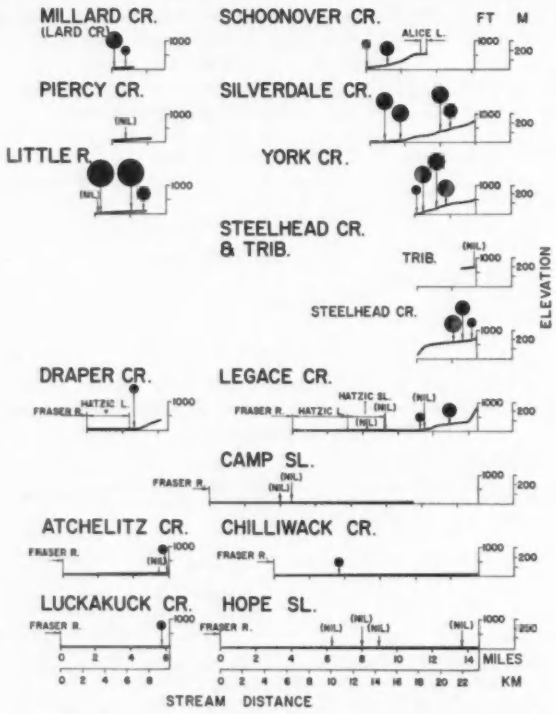

FIG. 5. Profiles of medium and low gradient streams with drainage areas under 120 km^2 in which all identifiable trout were cutthroat. Sampling stations, sample size, and composition are indicated. See Fig. 3 for legend.

stretches of stream. The micro-habitats of the two species appear to be similar although the major gradient characteristics of the stream they select are different.

In streams with drainage areas under 120 km^2 which supported both species of trout, those with relatively steep gradients, such as Cougar, Hunts, Brohm, and Weaver creeks (Fig. 7), were occupied predominantly by steelhead. On the other hand where collections were predominantly cutthroat, as in Kanaka, French, Pattison and Blaney creeks (Fig. 7), the profiles level at the downstream ends, where the creeks run through sloughs.

In the stream systems where both species occurred, cutthroat were the predominant species in upstream collections in 10 of 13 cases. Differences in distribution of steelhead and cutthroat along the length of a stream are best illustrated in the Salmon River, where trout collections were predominantly steelhead in the lower part of the main stem of the stream, and predominantly cutthroat in the upper part of the main stem and in the tributaries (Fig. 7).

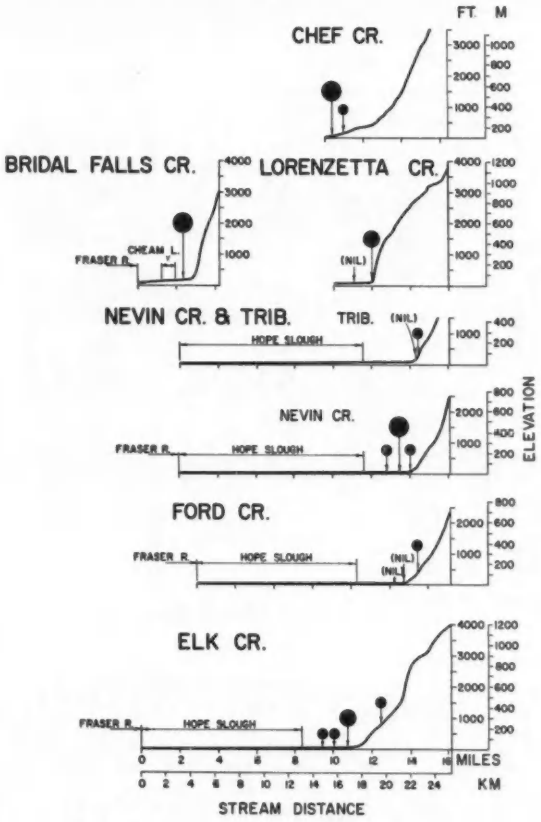

FIG. 6. Profiles of steep gradient streams which enter sloughs or level out in downstream areas. Drainage areas are under 120 km^2. All identifiable trout were cutthroat. Sampling stations, sample size, and composition are indicated. See Fig. 3 for legend.

EFFECT OF *p*H AND DISSOLVED SOLIDS

Data on *p*H for the two periods (November 1 to March 31 and April 1 to October 31) are not strictly comparable because numbers of readings and stations chosen were not all replicated. However, the *p*H values were generally higher during the April to October period than from November to March (Fig. 8). In the April to October period most of the *p*H values for streams with steelhead only, cutthroat only, and the species mixed, fell within the same range. During the November to March period, streams occupied by steelhead only had a narrower range of *p*H's than those occupied by cutthroat only. However, mean values for *p*H were near 7.00 for both groups of streams. The range and mean of *p*H values for streams in which both species occurred were slightly lower than for streams which supported only one species.

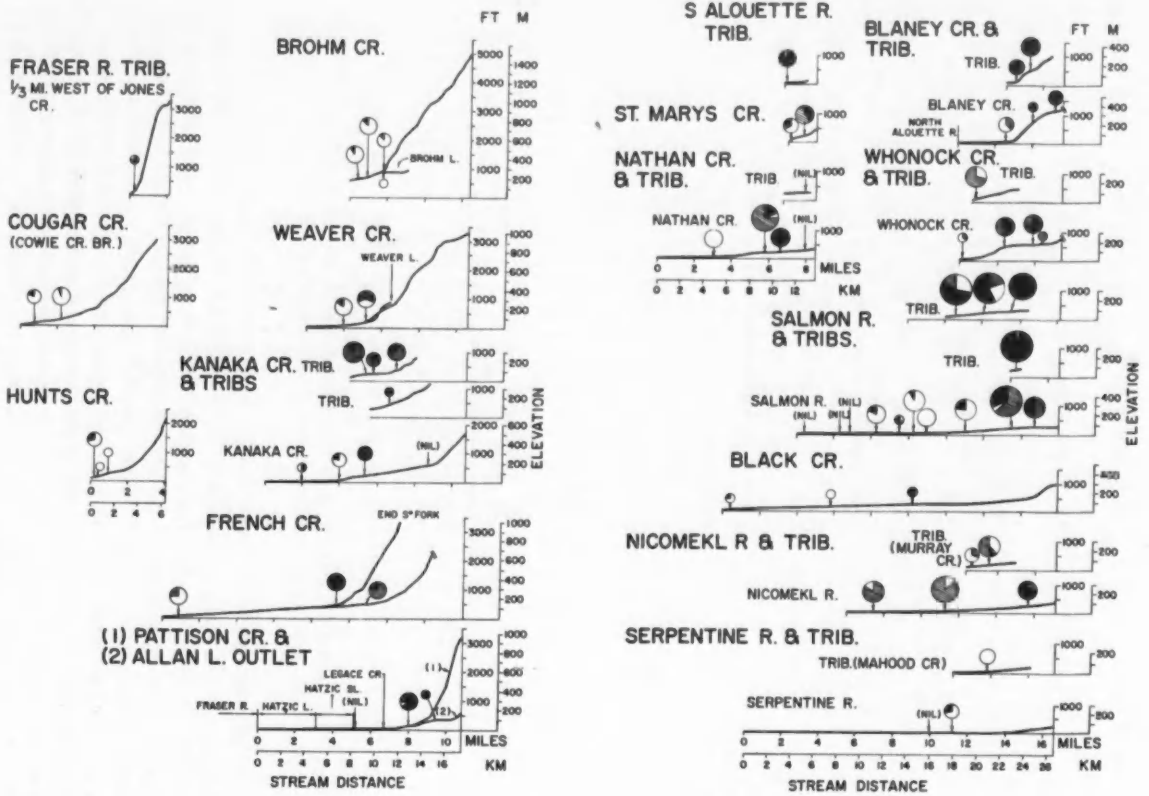

FIG. 7. Profiles of streams with drainage areas under 120 km² in which both steelhead and cutthroat occurred. Sampling stations, sample size, and composition are indicated. See Fig. 3 for legend.

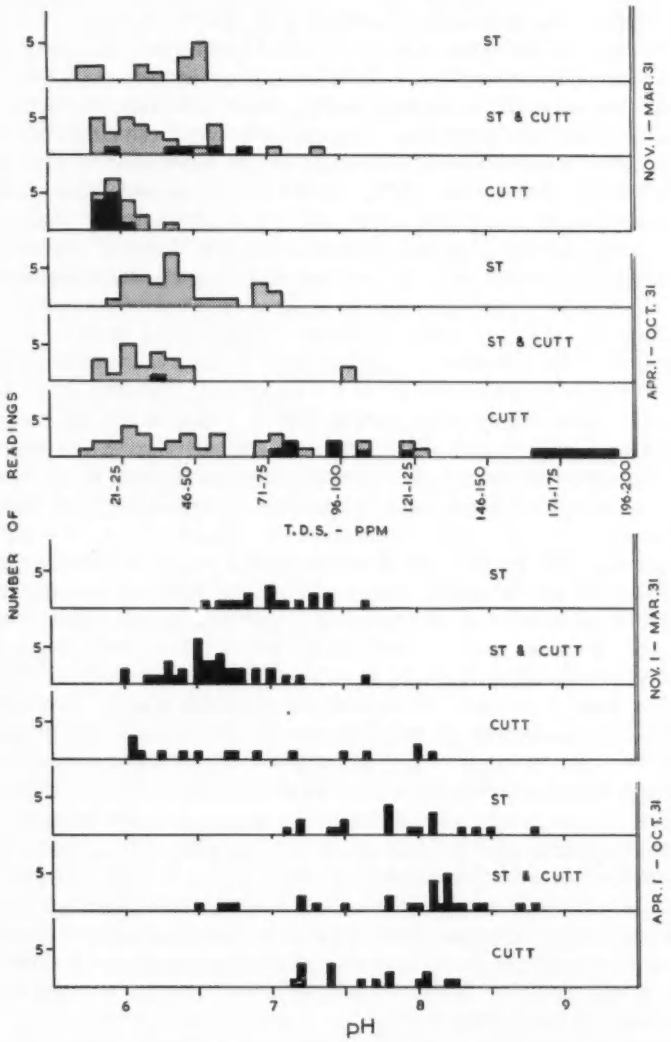

FIG. 8. Total dissolved solids and *p*H in streams with steelhead alone, both cutthroat and steelhead, and cutthroat alone. T.D.S. values indicated by black bars are from slough areas of streams.

Total dissolved solids values in the various streams ranged from 15 to 192 ppm in the April to October period and from 15 to 95 ppm in the November to March period (Fig. 8). During the November to March period virtually all the readings for streams with species both separate and mixed fell between

15 and 80 ppm. During the April to October period all the values fell between 16 and 105 ppm in the streams with steelhead alone and steelhead and cutthroat together. However, many T.D.S. readings in streams in which only cutthroat were obtained were high in the April to October period. Most of these high values were obtained in the slough areas of the cutthroat streams. Readings taken in corresponding locations in the November to March period were much lower (black bars in Fig. 8). Cutthroat streams were thus somewhat different from other streams in that their conductivity characteristics in many cases exhibited greater seasonal change. Neither *p*H or T.D.S., however, appeared to have any clear effect in limiting the distribution of either species.

DISCUSSION

A considerable degree of variability in the types of stream occupied by *Salmo gairdneri* and *S. clarki clarki* leads to some overlap in the distribution of the two species. Whereas large and steep streams support mainly steelhead and small and level streams mainly cutthroat, both species were found in a number of streams which were intermediate in drainage area (Serpentine and Nicomekl rivers and French and Kanaka creeks, Fig. 7) or profile (Kanaka, Cougar, Weaver, and Whonock creeks, Fig. 7). In some cases steelhead enter small streams which are not characteristic of streams usually chosen by them (Blaney and St. Mary's creeks, Fig. 7).

The occurrence of cutthroat in certain streams may be related to their existence in nearby lakes in the system, e.g. Brohm Creek, Puntledge and Quinsam rivers. Cutthroat juveniles are known to move down through fish ladder facilities at the outlet of Comox Lake. This may account for their occurrence in the lower Puntledge River. Lakes in the Quinsam River system may contribute cutthroat to the river. The occurrence of cutthroat in the downstream collection in the South Alouette River (Fig. 3) is almost certainly related to their localized presence in the small tributary (Fig. 7) which enters the main river at that location. Trout sampled a few meters up the small tributary of the South Alouette River (Fig. 7) are predominantly cutthroat. In view of the foregoing, it seems likely that the occurrence of cutthroat in the large streams and in Brohm Creek represents special, though not rare, circumstances for the species.

Previous hatchery introductions as early as 1930 may have altered the distributional relationships of rainbow and cutthroat. Cutthroat were introduced into the Nicomekl, Serpentine, and Salmon rivers and Kanaka Creek in 1933, according to British Columbia Game Department records. Many fish plantings in the past have not been well documented; therefore, it is not clear to what extent past introductions of fish might affect present distributions. However, since introductions to occupied stream habitats are generally not successful, it is unlikely that the distributional characteristics of cutthroat have been seriously confounded by these plantings.

It was beyond the scope of the study to distinguish migratory and resident races of trout. But it was noted that resident populations of cutthroat occur in a tributary of Blaney Creek. Females 111–132 mm long contained eggs which were nearly mature. Small, sexually mature male trout were taken in a number of streams. Steelhead Creek, which contains cutthroat trout in abundance, is not accessible to fish from the Stave River into which it flows. Presumably Steelhead Creek fish are resident in the stream system. Sumner (1952) reports the occurrence of resident cutthroat in migrations in Sand Creek, Oregon. Neave (1949) reports coastal populations of cutthroat trout above impassable falls and in small tributaries in the Cowichan River system, indicating further the occurrence of some non-migratory forms. Neave (1949) also reports coastal populations of resident *Salmo gairdneri*. There was less evidence of residence among *S. gairdneri* in the present study: few small ripe male fish were obtained. The existence of non-migratory fish is pointed out because the role of migration in determining distribution is speculated upon later in this discussion. Although the size and age of most fish sampled suggest that the juvenile coastal trout are largely progeny of migratory forms, some distribution patterns in stream systems are more extensive because of resident forms.

The stream *p*H values did not bear obvious relationship to differences in trout distribution. The data gathered from April to October indicate that stream systems used by cutthroat have greater gradients in T.D.S. (conductivities increase greatly in meadowland and slough areas) (Fig. 8) than those used by steelhead. Although data on *p*H and conductivity do not shed light on the differences in trout distribution in this study, they should not be ruled out as having no effect. Conductivity, *p*H, and probably other features of stream limnology relate to gradient and size. Such features may differ in various stream systems, and hence affect fish, in fashions not indicated by a superficial series of measurements made over all systems.

The existence of ecological zones and specific faunistic groups within streams can be related directly and indirectly to gradient characteristics (Huet, 1959, 1962; Burton and Odum, 1945). Although many British Columbia coastal streams contain zones with different faunas, the distributions of the two species of *Salmo* do not depend only on a stream slope–breadth relation. As already pointed out, juvenile cutthroat occur as a minor species in large streams with drainage areas over 130 km^2 (50 miles^2). Within streams below this size both species occur over a wide range of stream slopes (Fig. 9).

Although the estimations of stream width are extremely rough, the data for British Columbia indicate a high slope–breadth relationship among the smaller streams. The difference between these high values and the curve given by Huet (1959) probably represents a difference between local trout (steelhead and cutthroat) and brown trout as well as a difference in topography.

The micro-habitats in which *Salmo gairdneri* and *S. clarki* are found are very similar; these areas include pools, gravel riffles and runs, rocky turbulent stretches and plunge pools in white water torrent areas. In many streams the

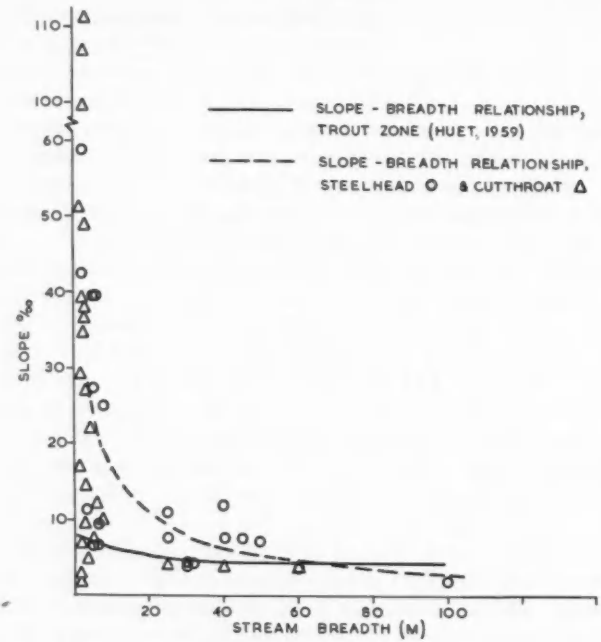

FIG. 9. Approximate relationship between stream gradient and breadth in British Columbia coastal trout streams and in the trout (*Salmo trutta*) zone of western European streams. Data for steelhead and cutthroat are for streams both above and below 130 km^2 in drainage area.

differences in species composition appear to be related to stream conditions below the areas where the fish occur. Such a situation leads to the speculation that differences in juvenile trout distribution may be related directly to differences in the migratory patterns of adults. Adult cutthroat trout presumably enter slough systems, slow-moving streams in meadowland areas, and small tributaries. The much larger adult steelhead, on the other hand, remain in larger streams or enter preferably into fast-flowing tributaries. At present, little comparative information on the migratory responses of the two species is available to support or contradict the idea that differences exist in their migratory behavior or choice of spawning areas.

Although sample sizes were small in some streams and collections were not numerous in others, the evidence that is available strongly indicates that stream size and profile affect trout distribution either directly or indirectly.

The implications of differences in distribution of juvenile steelhead and cutthroat trout in management are significant. In general the small cutthroat streams which run through farmland or sloughs are vulnerable to relocation,

straightening, obstruction, and pollution. Fish habitat in the steeper regions of the streams may be destroyed by scouring and debris accumulation as a result of logging activities over a small area of the watershed. Steelhead trout growing in small streams are vulnerable to the same influences as cutthroat. Many steelhead trout, however, dwell in larger rivers, which are less susceptible to local influence or destruction.

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Mr J. Chatwin prepared the final figures. His efforts and comments on the figures are much appreciated as are those of Mr R. Thomas.

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Influence of Dissolved Oxygen and Carbon Dioxide on Swimming Performance of Largemouth Bass and Coho Salmon[1,2,3]

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ABSTRACT

The final swimming speed of juvenile largemouth bass, *Micropterus salmoides* (Lacépède), was reduced markedly at oxygen concentrations below 5 or 6 mg/liter in tests at 25 C in a tubular chamber in which the velocity of water was increased gradually, at 10-min intervals, until the fish were forced by the current permanently against a screen. At levels above 6 mg/liter, the final swimming speed was virtually independent of the oxygen concentration. The performance of bass that had been acclimated overnight to elevated carbon dioxide levels was not materially affected by the highest tested concentrations of free carbon dioxide, averaging 48 mg/liter, at any tested level of dissolved oxygen.

For juvenile coho salmon, *Oncorhynchus kisutch* (Walbaum), at temperatures near 20 C and carbon dioxide concentrations near 2 mg/liter, any considerable reduction of the oxygen concentration from about 9 mg/liter, the air-saturation level, resulted in some reduction of the final swimming speed. The performance of the salmon was impaired much more markedly than was that of the bass by the same reduction of the oxygen concentration. At oxygen concentrations near and above the air-saturation level, high concentrations of free carbon dioxide averaging 18 and 61 mg/liter had a depressing effect on the final swimming speed of coho salmon even after overnight acclimation. However, this effect decreased at reduced oxygen concentrations. No measurable effect of free carbon dioxide concentrations near 61 mg/liter was evident at 2 mg/liter dissolved oxygen, and concentrations near 18 mg/liter had little or no effect even at moderately reduced dissolved oxygen levels after overnight acclimation of the salmon to these carbon dioxide concentrations.

INTRODUCTION

THE LITERATURE dealing with the influence of dissolved oxygen on the swimming performance of fishes has been reviewed recently by Doudoroff and Warren (1965), along with much other pertinent literature. Davis et al. (1963) found that even slight reduction of the dissolved oxygen concentration from the air-saturation level usually resulted in some reduction of the maximum sustained swimming speeds of juvenile coho salmon, *Oncorhynchus kisutch*, and chinook salmon, *O. tshawytscha*, at temperatures of 10–20 C. Katz et al.

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(1959) tested the ability of juvenile largemouth bass, *Micropterus salmoides*, 63–93 mm in total length to swim for 1 day against a current at a speed of about 24 cm/sec. In September, at 25 C, the bass were able to resist the current even when the oxygen concentration was only about 2 mg/liter, but in December, at temperatures of 15.5–17 C, they apparently were unable to do so when the oxygen concentration was reduced to 5 mg/liter. The maximum sustained swimming speeds of the bass possible under the various conditions were not determined.

Carbon dioxide has long been known to be toxic to fish (Doudoroff and Katz, 1950) and to reduce the affinity of their blood for oxygen at high as well as reduced oxygen tensions (Root, 1931). Basu (1959) measured the "active" oxygen consumption rates of four species of fish at various concentrations of both dissolved oxygen and carbon dioxide. Steady activity was ensured by mild electrical stimulation whereby the fish were forced to swim in a rotating annular respirometer. Basu found that the logarithm of the oxygen uptake rate declined linearly with increase of carbon dioxide concentration. This decline was much steeper at very low dissolved oxygen levels than at higher levels in the three species tested at the very low levels: the brown bullhead, *Ameiurus nebulosus*; carp, *Cyprinus carpio*; and goldfish, *Carassius auratus*. We know of no comparable experiments on the influence of free carbon dioxide on the swimming speeds of fish at various levels of dissolved oxygen.

The resistance to dissolved oxygen deficiency of resting coho salmon (McNeil, MS, 1956), largemouth bass (Hart, 1945, 1957), and many other species of fish has been found not to be markedly and lastingly reduced at carbon dioxide concentrations below 50 mg/liter. In experiments with coho salmon, the minimum effective carbon dioxide concentrations have been found to vary with the degree of rapidly progressing acclimatization or adjustment of the fish to the elevated carbon dioxide levels (McNeil, MS, 1956). They may also vary somewhat with the bicarbonate alkalinity of the water (McNeil, MS, 1956) and with temperature, but Doudoroff (1957) has pointed out that the wide variation with temperature of effective carbon dioxide *tensions* that has been reported (Fry et al., 1947) does not signify material variation of effective carbon dioxide *concentrations*. Alabaster et al. (1957) found that carbon dioxide concentrations well below 50 mg/liter had a marked effect on the dissolved oxygen requirements of rainbow trout, *Salmo gairdneri*. However, in their tests, the fish evidently were subjected to low oxygen concentrations and high carbon dioxide concentrations suddenly and simultaneously, so that they had no opportunity to become adapted to the elevated carbon dioxide levels before being subjected to critical or lethal dissolved oxygen levels.

The main objective of the present work was evaluation of the influence of carbon dioxide at high, low, and intermediate oxygen concentrations on the maximum sustained swimming speeds of largemouth bass at 25 C and of coho salmon at 20 C. Our initial undertaking, however, was accurate determination,

in late spring and early summer, of the influence of dissolved oxygen on the swimming ability of the bass at 25 C and at a low level of free carbon dioxide.

MATERIALS AND METHODS

Experimental Fish

The largemouth bass used in these studies were seined from a pond near Junction City, Oregon. Juvenile coho salmon were seined periodically from tributaries of the Alsea River in Benton County and from the Yaquina River in Lincoln County, Oregon. All fish were graded according to size; the largest and smallest fish were discarded. The mean total length of the bass used was 82 mm; the standard deviation was 2.3 mm. The mean total length of the coho salmon was 82 mm; the standard deviation was 3.9 mm.

The fish were kept in a constant temperature room in 50-gal (189-liter) glass aquaria supplied with running water from a small spring-fed stream. The largemouth bass were held at 25 C, the test temperature, and fed unrestricted rations of small, live earthworms for at least 18 days before use in experiments. Losses of bass in the stock tank were negligible during the experiments. The first lot of coho salmon used was held at 20 C, the test temperature, but heavy losses occurred which were due to an unidentified disease. The use of this lot of fish was discontinued when the disease became apparent and only a small number of the fish had been used. Subsequently, all the coho salmon were held at the lower acclimation temperature of 17 C for at least 5 days before use in experiments, and no more diseased fish were observed. The coho salmon were fed unrestricted rations of tubificid worms during the holding periods.

Experimental Apparatus

The experimental apparatus (Fig. 1) was designed for subjecting fish to a rectilinear flow of water of controlled velocity, temperature, and dissolved gas content in a glass tube (test chamber) 1.5 m long and 100 mm in inside diam. It has been described in detail by Davis et al. (1963) and diagrammed by Katz et al. (1959). Filtered stream water, supplied through polyethylene pipe and Tygon tubing, could be circulated continuously, by means of an all-iron centrifugal pump, through the tubular test chamber and through an aluminum heat exchanger immersed in a cooling bath for temperature control. Water velocities in the chamber could be adjusted by means of a gate valve located at the pump's outlet. The valve had an attached graduated dial and an indicator on the handwheel. It was calibrated with a small current meter.

The water in the system was renewed continuously at a rate of 1 liter per minute. Dissolved gas concentrations were maintained by introducing a gas, or gases, at the bottom of a vertical glass column filled with ceramic Raschig rings, through which the water flowed downward before entering the experimental chamber. Nitrogen, compressed air, and oxygen were used for maintaining concentrations of dissolved oxygen below, at, and above air-saturation

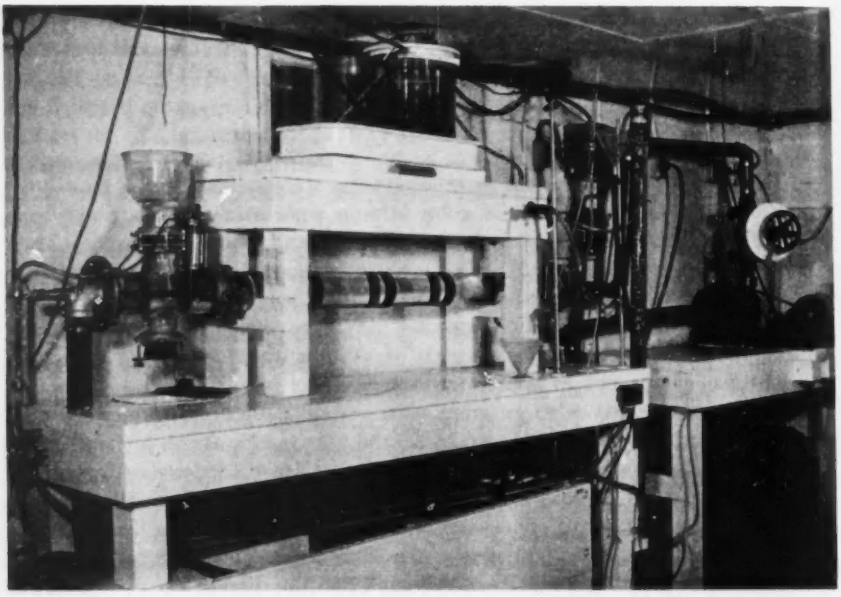

FIG. 1. The experimental apparatus.

levels, respectively. Carbon dioxide was used in combination with the above gases to attain concentrations of this gas above the low level characteristic of the untreated water.

A few minor changes were made in the apparatus described by Davis et al. (1963). A fixed aluminum screen was added behind the movable (retractable) screen at the downstream end of the test chamber, behind the fish-introduction port and funnel. This enabled removal of each fish from the experimental chamber as soon as it was forced by the current against the movable screen, without danger of the fish being swept out of the chamber and through the pump. The control and measurement of the flow of water through the vertical glass column and into the test chamber were facilitated by fitting a ball-displacement flowmeter and a glass stopcock to the water supply tube between the column and the chamber. Bands of black plastic tape were attached to the outside of the test chamber wall as an aid to visual orientation of the fish.

EXPERIMENTAL PROCEDURE

The procedures followed in experiments with the two species of fish were essentially the same. Five fish of uniform size were taken from the holding tank the day before a test and placed in the test chamber late in the afternoon, the temperature within the apparatus having been previously adjusted. As

soon as the fish were calm, the water velocity was increased to 7.6 cm/sec. At this low water velocity, the fish were able to maintain their positions within the chamber with little or no exertion. The fish were left undisturbed in the apparatus until the next morning (about 16 hr), when the water velocity was gradually increased, in 5 or 15 min, to a base velocity of 15.2 cm/sec in the experiments with largemouth bass and 22.8 cm/sec in those with coho salmon. These two base velocities, especially the first, were not much above the lowest velocities at which the fish had to swim to maintain their positions within the test chamber and avoid impingement on the downstream closure. After the desired water velocity had been reached, the dissolved oxygen concentration was reduced or increased as necessary. The dissolved oxygen content of the water in the chamber reached the desired constant level and the fish learned to swim steadily against the current during a subsequent 4-hr period, after which the water velocity was raised by uniform increments until all five fish failed to continue swimming. Both largemouth bass and coho salmon were subjected to velocity increments of 2.3 cm/sec every 10 min, starting in each case from the stated base velocity.

The free carbon dioxide concentration was adjusted at the same time as the dissolved oxygen concentration in one series of tests with each species of fish. In all other experiments in which the fish were subjected to elevated levels of free carbon dioxide, the carbon dioxide concentration was adjusted before placing the fish in the experimental chamber, but the dissolved oxygen concentration remained near the air-saturation level until the following morning.

In tests with coho salmon, a beam of light from a 60-w incandescent lamp was directed at the downstream end of the chamber to discourage fish from swimming in the immediate vicinity of the movable screen. The downstream closure (retractable screen) was rotated as necessary in order to stimulate all fish that had failed to continue swimming and had come to rest against the closure. The water velocity at which an individual fish apparently had been forced permanently against the screen was recorded as the *final swimming speed* of that fish. The fish was removed with the aid of a small dip net inserted into the test chamber through the funnel-like receptacle and port at the downstream end of the chamber; retraction of the movable screen permitted the current to carry the fish off the screen and into the dip net. After removal, the fish were held separately in 1-liter beakers until termination of the test.

Water temperatures were checked frequently during experiments. The temperature ranges in experiments with coho salmon did not exceed 1.5 degrees C with but four unimportant exceptions. The ranges tended to be greater in the earlier tests with largemouth bass, exceeding 1.5 but not 2.3 degrees C in eight tests, and failures of the thermoregulatory system in two experiments resulted in 3.6 and 2.8 degrees C fluctuations. After the last fish to fail had been removed from the test chamber, samples of water flowing out of the test chamber were taken for chemical analyses. All water analyses were done by standard methods (American Public Health Association, 1960). Dissolved oxygen concentrations near or below the air-saturation level were determined

by the Alsterberg (azide) modification of the Winkler method; the Pomeroy–Kirschman–Alsterberg method was used for determining concentrations far above the air-saturation level. Carbon dioxide levels were determined nomographically, after determining *p*H, total alkalinity, temperature, and total dissolved solids. After the water analyses had been completed, the fish were individually measured and weighed, and blood samples usually were taken for hemoglobin determinations.

The hemoglobin content of the blood of the test animals was determined at the ends of most of the tests. The cyanmethemoglobin method, as described by Wintrobe (1961), was employed in all determinations. A 20-lambda sample of blood, obtained by severing the caudal peduncle of a fish, was diluted in 5 ml of Drabkin's solution, which converted the hemoglobin to cyanmethemoglobin; the latter was measured electrophotometrically. A Beckman Model D.U. spectrophotometer was used to measure the optical density of the cyanmethemoglobin solution at a wave length of 540 mμ. The optical density scale of the spectrophotometer was periodically standardized, using Hemotrol as a hemoglobin standard.

EXPERIMENTS ON LARGEMOUTH BASS

Experiments with largemouth bass (Table I) were performed between April 25 and July 25, 1962. All mean weights, and final swimming speeds in Table I are means for five fish except those pertaining to only one test. In that test, the first-failing fish had an injured caudal fin; therefore, the values for the other four fish only were averaged. Data on the individual fish, and also ranges of water temperatures observed during the tests, have been reported by Dahlberg (MS, 1963).[5] The final swimming speed of a fish in lengths per second (L/sec) is a relative speed calculated by dividing the recorded final swimming speed, expressed in centimeters per second, by the total length of the fish in centimeters.

The use of relative swimming speeds of bass (L/sec), rather than absolute speeds (cm/sec), appeared to be decidedly advantageous. The absolute speeds of bass tested at high oxygen and low carbon dioxide concentrations tended to increase with increase of body length. The relative speeds (L/sec), on the other hand, appeared to be independent of body length within the narrow range of lengths represented (75–88 mm).

It is apparent (Fig. 2) that the swimming speed of largemouth bass at low carbon dioxide concentrations decreased with reduction of dissolved oxygen concentration below 5 or 6 mg/liter. At tested oxygen concentrations above 6 mg/liter, the performance of bass apparently was virtually independent of oxygen concentration.

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[5]Some errors to be found in Dahlberg's (MS, 1963) thesis, such as inaccurate determinations of free carbon dioxide levels, were corrected in the course of preparation of the present paper through reference to original (bench) notes and recomputation.

TABLE I. Experimental conditions and the mean sizes and final swimming speeds of juvenile largemouth bass (usually five) in tests at various concentrations of dissolved oxygen and carbon dioxide.

Date of expt.	Mean temp (*C*)	Mean dissolved oxygen (*mg/liter*)	Free carbon dioxide[a] (*mg/liter*)	Total alkalinity[b] (*mg/liter*)	*p*H	Mean total length (*mm*)	Mean weight (*g*)	Mean final swimming speed	
								cm/sec	*L/sec*
4/25/62	24.7	8.40	–	–	–	80.8	4.86	37	4.6
4/26/62	25.1	5.20	–	–	–	81.8	5.52	40	4.9
4/27/62	25.2	2.20	–	–	–	83.0	6.04	33	4.0
4/28/62	25.1	6.40	–	–	–	84.6	6.20	41	4.9
4/29/62	24.8	13.30	–	–	–	84.0	5.54	38	4.5
4/30/62	25.2	1.40	–	–	–	81.2	5.24	25	3.1
5/1/62	25.1	3.50	–	–	–	80.4	5.31	36	4.5
5/5/62	24.9	11.40	–	–	–	82.4	6.10	41	5.0
5/8/62	25.1	1.00	–	–	–	82.0	5.95	20	2.4
5/9/62	25.0	19.70	–	–	–	81.6	5.78	40	5.0
5/10/62	24.9	4.50	–	–	–	80.8	5.69	38	4.7
5/11/62	24.9	3.00	–	–	–	81.4	5.68	36	4.4
5/14/62	24.9	10.30	–	–	–	82.8	6.03	39	4.7
5/15/62	25.2	4.30	–	–	–	83.2	6.36	36	4.4
5/16/62	25.1	2.50	–	–	–	81.2	5.80	31	3.8
5/17/62	24.9	6.60	–	–	–	81.4	6.01	37	4.6
5/18/62	24.8	1.50	–	–	–	82.2	5.66	27	3.3
5/23/62	25.0	24.00	–	–	–	82.8	6.39	37	4.7
5/24/62	25.1	7.60	–	–	–	81.8	5.72	37	4.5
6/26/62	25.0	8.10	2.5	85	7.80	79.6	5.58	40	5.0
7/4/62	25.1	4.70	–	–	–	81.2	5.81	38	4.6
7/5/62	25.0	3.10	–	–	–	81.2	5.94	36	4.5
7/10/62	25.0	7.90	25	94	6.85	82.8	6.11	40	4.8
7/11/62	24.9	3.30	38	92	6.65	81.8	6.13	37	4.5
7/12/62	25.3	2.10	24	–	6.85	82.2	6.31	35	4.2
7/13/62	24.9	1.35	28	94	6.80	81.8	6.45	29	7.8
7/16/62	24.9	5.10	26	96	6.80	86.2	7.36	43	5.0
7/17/62	23.4	1.85	44	–	6.60	83.0	6.20	30	3.7
7/18/62	24.8	7.85	43	96	6.60	83.0	6.32	41	4.8
7/19/62	24.8	3.20	49	–	6.55	83.6	6.25	38	4.5
7/20/62	24.8	5.10	54	–	6.50	83.6	6.41	40	4.7
7/24/62	25.0	2.10	18*	–	7.00	81.8	5.82	32	3.9
7/25/62	24.1	1.20	23*	97	6.90	83.4	6.11	24	2.8
7/26/62	25.1	4.40	22*	–	6.90	85.2	6.82	40	4.7

[a]An asterisk (*) indicates that fish were not acclimated to the carbon dioxide concentration shown. A dash (—) indicates that carbon dioxide was not added to the stream water and the low concentration of free CO_2 in the water was not evaluated (*p*H not determined).

[b]Bicarbonate alkalinity expressed as calcium carbonate ($CaCO_3$) equivalent. Determinations of total dissolved solids ranged between 130 and 133 mg/liter.

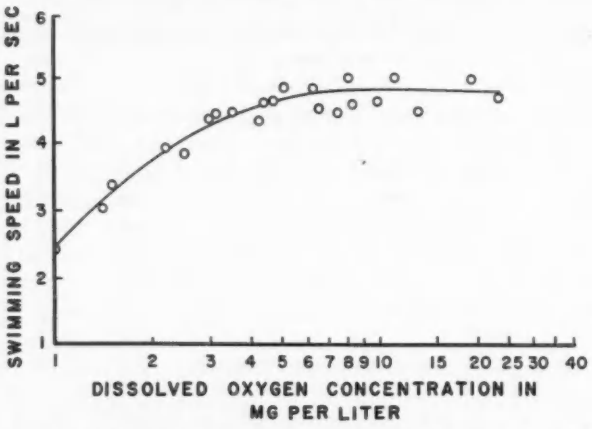

FIG. 2. Relationship between the mean final swimming speed of largemouth bass, expressed in L/sec, and the dissolved oxygen concentration at nearly natural, low concentrations of carbon dioxide (probably <3 mg/liter) and temperatures near 25 C.

Free carbon dioxide concentrations were not determined during the tests whose results are plotted in Fig. 2, with but one exception. Determinations made under similar experimental conditions during the same season of the next year (the spring of 1963) indicated that free carbon dioxide concentrations probably never exceeded 3 mg/liter during the test period.

Figure 3 facilitates comparison of the results of the different kinds or groups of experiments performed with largemouth bass. In the group of tests in which the concentrations of free carbon dioxide averaged 21 mg/liter, the fish were exposed to them for little time before the swimming performance trials, the carbon dioxide concentration having been increased at the same time that the dissolved oxygen concentration was reduced. The data to which the curve was fitted are not shown in Fig. 3, except three points (solid triangles) that represent data obtained later than the rest and at about the time that the tests at elevated carbon dioxide concentrations were performed. The individual test data from the latter experiments are all included in Fig. 3, and most of them do not deviate markedly from the curve. Those that do deviate considerably are above the curve, and not below, indicating improvement of swimming performance. However, since all of the three points based on tests at low carbon dioxide concentrations (solid triangles) also fall above the curve, it appears that the bass probably were generally able for some reason to perform slightly better in the later tests than in the earlier ones under the same conditions. Thus, it can be concluded that concentrations of free carbon dioxide even above 40 mg/liter, which are rarely found in nature, had virtually no adverse effect on the swimming performance of bass, but there is not sufficient evidence of improvement of the performance at any concentration of carbon dioxide.

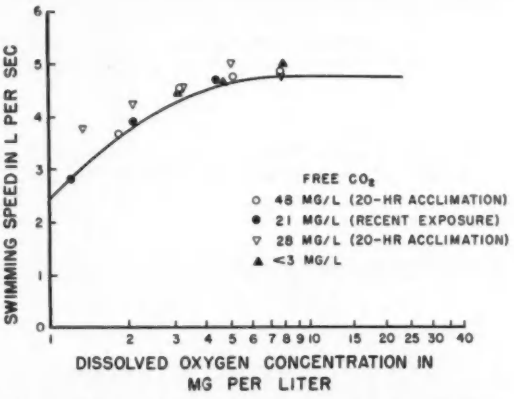
SWIMMING SPEED IN L PER SEC
DISSOLVED OXYGEN CONCENTRATION IN MG PER LITER
FREE CO_2
48 MG/L (20-HR ACCLIMATION)
21 MG/L (RECENT EXPOSURE)
28 MG/L (20-HR ACCLIMATION)
<3 MG/L

FIG. 3. Mean final swimming speeds, expressed in L/sec, of largemouth bass at various concentrations of dissolved oxygen and carbon dioxide. The curve relating swimming speed to oxygen concentration is the curve that was fitted to all data from tests at the low concentration of free carbon dioxide (probably <3 mg/liter) only, most of which (those from early tests) have not been plotted here. The stated free carbon dioxide levels of 48, 21, and 38 mg/liter are means of observed values for individual tests ranging from 44 to 55 mg/liter, 17 to 23 mg/liter, and 24 to 38 mg/liter, respectively. See text for further details.

There was not much difference in performance between the first-failing and third-failing bass, and the data pertaining to each do not deviate widely from the respective eye-fitted curves (Fig. 4).

Hemoglobin determinations were made on blood samples from 67 juvenile largemouth bass used in the performance tests, none having been made in the earliest tests. The mean and standard deviation of the values obtained (Dahlberg, MS, 1963) are 7.92 and 0.47 g/100 ml, respectively.

EXPERIMENTS ON COHO SALMON

Experiments with coho salmon (Table II) were performed between August 6 and November 9, 1962, and between October 20 and December 10, 1964. Data on individual fish tested in 1962 have been reported by Dahlberg (MS, 1963).[6] Five fish were used in each test and the final swimming speed (also length and weight) was recorded for each of the five fish except in seven tests in which only four terminal speeds were recorded, and one test in which three final speeds were noted. In seven of the excepted tests, one or two fish (two

[6]As noted earlier, some errors to be found in Dahlberg's (MS, 1963) thesis, such as inaccurate determinations of free carbon dioxide, were corrected in the course of preparation of the present paper. These errors include the recording of the fork lengths of coho salmon used in experiments No. 61 through 77 as total lengths.

TABLE II. Experimental conditions and the mean sizes and final swimming speeds of juvenile coho salmon (usually five) in tests at various concentrations of dissolved oxygen and carbon dioxide. See text for further details.

Date of expt.	Mean temp (*C*)	Mean dissolved oxygen (*mg/liter*)	Free carbon dioxide[a] (*mg/liter*)	Total alkalinity[b] (*mg/liter*)	*p*H	Mean total length[c] (*mm*)	Mean weight (*g*)	Mean final swimming speed		Mean adjusted final swimming speed[d] (*L/sec*)[e]
								cm/sec	*L/sec*[e]	
8/6/62	20.1	9.50	1.9	–	8.00	84.4	4.97	54	6.4	6.5
8/7/62	19.8	4.75	2.6	95	7.85	83.2	4.65	51	6.1	6.2
8/9/62	20.0	9.60	58	–	6.50	85.6	4.99	44	5.2	5.3
8/10/62	20.0	4.60	58	95	6.50	80.6	4.51	41	5.1	5.0
8/13/62	20.0	17.50	1.7	–	8.05	80.0	4.63	58	7.2	7.1
8/14/62	20.1	6.10	1.4	103	8.15	81.6	4.71	54	6.6	6.6
8/15/62	19.9	11.70	1.4	103	8.15	92.0	6.62	60	6.5	7.0
8/16/62	19.7	3.40	1.5	103	8.18	86.6	5.59	39	4.5	4.7
8/17/62	20.0	25.70	1.5	102	8.18	83.8	4.99	58	6.8	6.9
8/20/62	19.9	2.20	1.5	104	8.18	84.0	4.89	32	3.8	3.9
8/21/62	20.0	2.70	45	100	6.65	81.6	4.70	36	4.4	4.4
8/22/62	19.9	24.90	50	–	6.60	80.8	4.43	49	6.1	6.0
8/23/62	20.0	6.80	40	101	6.70	77.8	3.96	49	6.4	6.2
8/24/62	20.0	7.20	1.6	–	8.10	85.0	5.14	61	7.1	7.3
8/27/62	20.3	2.10	2.1	100	7.95	84.8	5.09	28	3.3	3.3
9/4/62	20.3	2.10	41	103	6.68	85.2	5.51	31	3.6	3.7
9/5/62	20.0	15.30	46	–	6.65	92.6	6.93	56	6.1	6.6
9/6/62	20.0	6.10	18	–	7.05	88.6	5.86	56	6.3	6.6
9/7/62	20.0	9.20	18	–	7.05	82.0	4.77	57	7.0	7.0
9/8/62	20.0	4.50	18	–	7.05	80.6	4.40	50	6.2	6.2
9/9/62	20.0	22.80	18	–	7.05	81.6	4.53	58	7.0	7.0
9/10/62	19.9	3.70	17	104	7.10	80.6	4.36	45	5.6	5.5
9/11/62	19.9	2.80	2.5	–	7.90	79.6	4.29	39	4.9	4.9
9/14/62	20.1	20.15	2.3	–	7.95	77.6	3.97	60	7.7	7.5
9/15/62	20.0	5.20	2.3	–	7.95	80.4	4.43	54	6.6	6.6
9/16/62	20.1	2.00	2.0	–	8.00	86.4	5.23	33	3.8	3.9
9/17/62	20.0	7.50	2.0	–	8.00	85.2	5.13	64	7.4	7.5
9/18/62	20.1	10.35	2.5	–	7.92	88.8	5.86	66	7.4	7.7
9/19/62	20.1	4.20	2.8	100	7.85	86.0	5.09	54	6.3	6.5
9/22/62	20.0	15.20	20	100	7.00	84.0	4.97	57	6.8	6.9
9/23/62	20.3	2.00	16	102	7.10	81.5				
9/24/62										

9/18/62	20.1	10.35	2.5							
9/19/62	20.1	4.20	2.8	100	7.85	86.0	5.09	54	6.3	6.3
9/22/62	20.0	15.20	20	100	7.00	84.0	4.97	57	6.8	6.9
9/23/62	20.3	2.00	16	10[illegible]	7.10	81.5	4.63	32	3.9	3.9
9/24/62	20.2	9.60	18*	–	7.05	78.3	3.98	51	6.6	6.4
9/25/62	19.4	4.80	18*	–	7.05	81.6	4.00	47	5.7	5.7
9/28/62	20.0	3.70	2.2	80	7.85	81.0	4.33	43	5.3	5.3
9/30/62	20.1	17.00	18*	98	7.05	75.6	3.51	44	5.9	5.6
10/1/62	20.0	3.30	16*	–	7.10	77.4	3.92	41	5.2	5.1
10/2/62	20.0	6.90	16*	–	7.10	80.0	4.00	53	6.6	6.5
10/3/62	20.0	2.60	20*	–	7.00	76.8	3.65	36	4.7	4.5
11/2/62	20.0	3.80	2.0	–	8.00	79.8	3.99	47	5.8	5.8
11/3/62	20.0	13.90	18*	99	7.05	81.8	4.14	57	6.9	6.9
11/4/62	20.0	2.70	16	99	7.10	80.8	4.06	37	4.5	4.5
11/5/62	20.2	2.55	2.2	–	7.95	83.0	4.66	38	4.5	4.5
11/6/62	20.2	17.60	39	99	6.70	86.6	5.11	60	7.0	7.2
11/9/62	20.1	17.80	3.2	–	7.80	82.0	4.67	64	7.8	7.8
10/21/64	18.3	9.75	1.8	106	8.10	84.6	5.00	60	7.1	7.3
10/22/64	20.3	9.00	1.7	102	8.10	81.4	4.31	58	7.1	7.1
10/28/64	19.7	8.90	68	102	6.50	82.0	4.65	49	5.9	5.9
10/29/64	19.8	4.90	60	110	6.60	82.0	4.48	47	5.9	5.9
10/30/64	20.0	5.05	2.2	102	7.95	81.4	4.25	52	6.4	6.4
11/3/64	20.2	2.78	2.2	92	7.95	82.8	4.77	39	4.7	4.7
11/4/64	20.1	2.80	55	88	6.55	82.0	4.61	35	4.3	4.3
11/6/64	19.9	14.70	65	98	6.40	82.8	4.75	51	6.1	6.2
11/7/64	20.1	18.10	1.5	–	8.00	81.8	4.75	61	7.4	7.4
11/12/64	20.0	19.80	62	74	6.35	82.0	4.58	54	6.6	6.6
11/13/64	20.0	2.25	70	–	6.40	82.0	4.80	33	4.0	4.0
11/17/64	20.2	2.30	2.0	94	7.95	82.0	4.73	35	4.3	4.3
11/20/64	20.0	24.10	64	100	6.50	80.0	4.34	53	6.6	6.5
11/24/64	19.8	6.55	120	80	6.20	82.2	4.80	50	6.1	6.1
11/25/64	19.8	24.45	3.8	56	7.60	82.0	4.65	57	7.0	7.0
12/1/64	19.9	6.40	2.2	40	7.50	81.5	4.56	58	7.1	7.1
12/4/64	20.1	12.50	3.0	50	7.50	82.0	4.82	62	7.5	7.5
12/8/64	20.0	11.20	95	52	6.10	82.2	4.50	54	6.5	6.5
12/10/64	20.1	21.50	66	54	6.20	80.4	4.61	51	6.3	6.2

[a]An asterisk (*) indicates that fish were not acclimated to the carbon dioxide concentration shown.

[b]Bicarbonate alkalinity expressed as calcium carbonate ($CaCO_3$) equivalent. Determinations of total dissolved solids ranged between 160 and 198 mg/liter.

[c]The total lengths (TL) of the salmon in mm were computed from the measured fork lengths (FL) by an appropriate conversion formula (TL = 1.0804FL + 0.13).

[d]Size-adjusted for a uniform total body length of 82 mm.

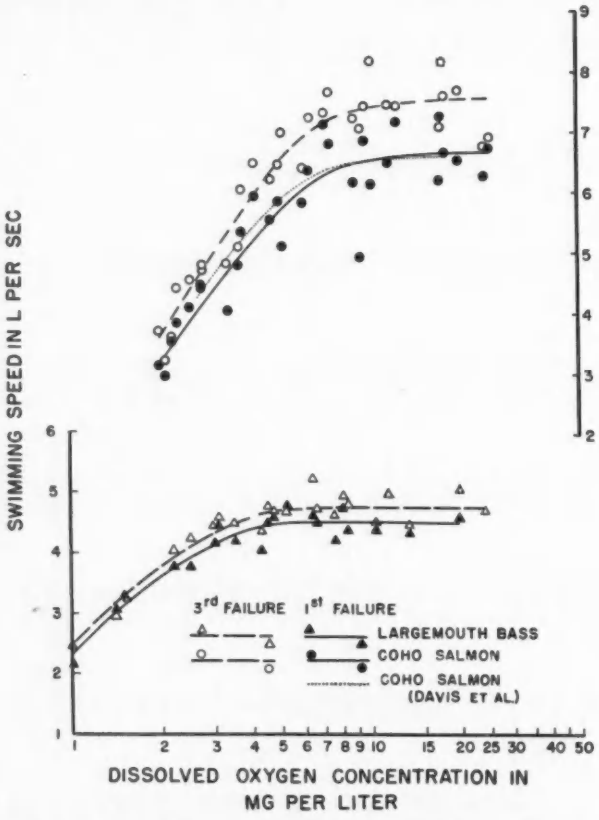

FIG. 4. Final swimming speeds, in L/sec, of first-failing and third-failing (of five) coho salmon and largemouth bass at low concentrations (<3 mg/liter) of carbon dioxide in relation to the dissolved oxygen concentration. The swimming speeds of the coho salmon were adjusted (size-corrected) for a total length of 82 mm in the manner explained in the text. The curve based on the data of Davis et al. (1963) is shown without the individual observations to which it was fitted. It relates the adjusted final swimming speeds at 20 C of first-failing coho salmon to oxygen concentration. Inasmuch as observations pertaining to failures at the base velocity are of questionable value (Davis et al., 1963), such an observation at an oxygen concentration of about 1.5 mg/liter was discarded in plotting the latter curve. All curves were fitted by eye.

in one test only) remained in a favorable position near the baffles at the upstream end of the test chamber. These fish were thus seemingly able to withstand water velocities much higher than the terminal velocities recorded for other fish tested. In one test a fish found to have frayed fins was disregarded. In most experiments the fish did not find the favorable positions described,

and all five final swimming speeds were used in computing the means. In experiments reported by Davis et al. (1963), chinook salmon often derived advantage from the eddies behind the baffles, but coho salmon did so only rarely.

The relative swimming speeds in L/sec were adjusted (size-corrected for a total body length of 82 mm) so as to compensate for difference of the fish in size only, which is known to influence both the absolute swimming speed (in cm/sec) and the relative speed (in L/sec). When the total length of a fish was greater than 82 mm (mean length of all the coho salmon used), the adjustment was made by taking the logarithm of the observed swimming speed of a fish in L/sec, adding a value equal to 0.0027 times the difference between 82 mm and the actual length of the fish in mm, and then finding the antilogarithm of the sum. When the length of the fish was less than 82 mm, a corresponding adjustment was made by subtraction.

According to unpublished data obtained in our laboratory by E. M. Smith, Jr., the logarithm of the final relative swimming speed of juvenile coho salmon in L/sec declines almost linearly by about 0.0027 with increase in total length of the fish by 1 mm within the length range of 70–120 mm. Our own data are in fair agreement with this finding, which is based on results of numerous tests at high oxygen and low carbon dioxide concentrations performed by Smith with our apparatus and by methods similar to ours. The above relationship does not hold for salmon much less than 70 mm in length, and it obviously cannot apply to fish much more than 120 mm long. Smith's data on sustained speeds do not agree well with the linear relationship suggested by Bainbridge (1960) between the logarithm of body length of a fish and the logarithm of the maximum absolute swimming speed sustainable for a relatively short time interval (burst speed). Bainbridge's method of speed adjustment for differences in body length was therefore rejected in favor of the above-described method. Within the range of lengths of our coho salmon (74–100 mm), the decrease of their relative sustained swimming speed (L/sec) with increase of body length is about as great, proportionally, as is the increase of the absolute speed (cm/sec). Thus, the computation and use of unadjusted relative speeds is not decidedly advantageous in the case of the coho salmon, as it appeared to be in the case of the largemouth bass. Nevertheless, it was decided to express adjusted speeds of coho salmon in L/sec, rather than in cm/sec, so that the data for salmon and bass plotted in our figures would be comparable.

In Fig. 5, the points representing results of the two tests at carbon dioxide concentrations above 70 mg/liter (95 and 120 mg/liter) are given as solid circles; the 16 others of the same group represent results of tests at concentrations ranging from 39 to 70 mg/liter and averaging 55 mg/liter. All but one of the tests at concentrations ranging from 39 to 58 mg/liter (mean 48 mg/liter) were performed in 1962, and all tests at concentrations of 60 mg/liter and more (mean 74 mg/liter) were performed in 1964. Surprisingly, there was no evident difference between the results obtained in these two series of tests, so that one curve fits all of these data reasonably well. Even

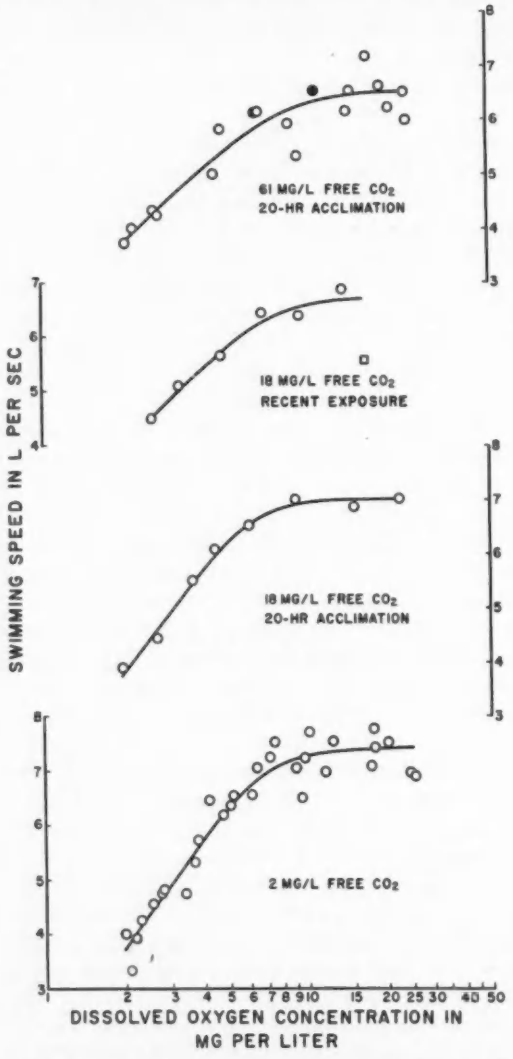

FIG. 5. Mean adjusted (size-corrected for a total length of 82 mm) final swimming speeds of coho salmon at different mean levels of free carbon dioxide in relation to dissolved oxygen concentration. The solid circles represent results of two tests at the very high carbon dioxide concentrations of 95 and 120 mg/liter. For fish recently exposed to 18 mg/liter free CO_2, the single square represents an anomalous result that was disregarded in fitting the curve to the other observations even though we are not aware of any fault of the test (at 17 mg/liter dissolved oxygen) that would account for the low mean final swimming speed of the fish. The stated free carbon dioxide levels of 61, 18, and 2 mg/liter are means of observed values for individual tests ranging from 39 to 120 mg/liter (with only two values above 70 mg/liter), 16 to 28 mg/liter, and 1.4 to 3.8 mg/liter, respectively. The curves were fitted by eye. See text for further details.

the two points obtained in 1964 at carbon dioxide concentrations of 95 and 120 mg/liter are close to the curve and slightly above it.

Between dissolved oxygen concentrations of about 2 mg/liter and 7 or 8 mg/liter, the swimming speed of the coho salmon declined markedly and almost linearly with decrease of the logarithm of the dissolved oxygen concentration (Fig. 5). Within the range of tested dissolved oxygen concentrations above 9 mg/liter, variation of oxygen concentration had only a slight effect on the swimming performance.

Figure 6 facilitates comparison of the curves shown in Fig. 5. Except at more or less reduced oxygen concentrations, elevated carbon dioxide concentrations near 18 mg/liter apparently influenced the final swimming speed

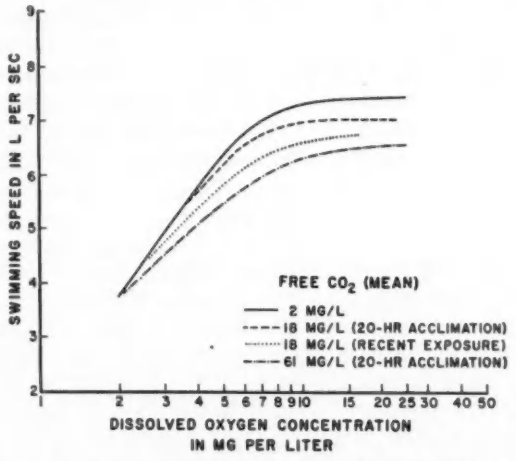

FIG. 6. Comparison of curves (from Fig. 5) relating mean adjusted (size-corrected for a total length of 82 mm) final swimming speeds of coho salmon to dissolved oxygen concentration obtained at different mean levels of free carbon dioxide.

of coho salmon, especially when the fish had not become adjusted to them. Even after overnight acclimation, free carbon dioxide concentrations ranging from 39 to 120 mg/liter and averaging 61 mg/liter evidently had a pronounced effect on the swimming speed, but only at dissolved oxygen concentrations well above 2 mg/liter. At the 2 mg/liter level of dissolved oxygen, no effect of the high free carbon dioxide concentration was apparent, and the greatest effect occurred at an oxygen concentration near and above 6 mg/liter. When oxygen concentrations were above 6.0 mg/liter, 11 of 12 tests at the highest carbon dioxide concentrations yielded mean adjusted final swimming speeds below 6.8 L/sec, whereas 14 of 16 tests at the lowest carbon dioxide concentrations yielded mean values above 6.8 L/sec (Fig. 5). Thus, there can be

little doubt that the difference between the curves obtained for the highest and lowest carbon dioxide concentrations (Fig. 6) is not fortuitous. The expected, intermediate positions of the two curves obtained at intermediate carbon dioxide concentrations averaging 18 mg/liter should be considered as confirmatory evidence.

A rather marked difference can be seen between the swimming speeds of first-failing and third-failing coho salmon (Fig. 4). There is very little difference between the curves based on first-failure data reported here and the first-failure data reported by Davis et al. (1963), who used underyearling coho salmon of about the same size as those used in our study and recorded first and second failures only. The differences between final swimming speeds of our first-failing and third-failing fish probably were due to persistent differences in swimming ability of individual fish like those found by Thomas et al. (1964) through retesting of groups of juvenile salmonids. Our fish were not retested, however, to determine whether or not the same individuals consistently show poor or good performance in repeated trials.

Hemoglobin determinations were made on blood samples from 184 coho salmon (in all but five tests) in 1962, but none in 1964. The mean and the standard deviation of the values obtained (Dahlberg, MS, 1963) are 6.85 and 0.82 g/100 ml, respectively.

DISCUSSION

The swimming speeds sustained by fish for 10 min that are reported here as final swimming speeds suitable for our comparative purposes are not necessarily speeds sustainable indefinitely. Brett (1964) has considered, with attention to pertinent earlier literature, the duration of tests necessary for measuring the true sustained swimming capacity of fish, as indicated by the relation between swimming velocity and fatigue time. On the basis of his own limited data, he concluded that "for sockeye [salmon] a reliable estimate of sustained speed could not be obtained under a test period of about 5 hr." More extensive unpublished data on coho salmon recently obtained in our laboratory by E. M. Smith provide no support for a 5-hr test period but considerable justification for a test period of about 30–50 min. However, they also show that the ability of an individual coho salmon to maintain a certain speed for an hour, or even for 5 hr, does not signify that its failure to maintain that speed cannot occur soon thereafter. Furthermore, practical considerations precluded the use of time intervals much greater than 10 min between velocity increments in our tests. The 10-min intervals somewhat arbitrarily chosen by Davis et al. (1963) therefore were adopted also in our work, facilitating comparison of our results with those of the latter authors. Having used 20-min time intervals between velocity increments instead of 10-min intervals in a few tests performed for comparative purposes, Davis et al. (1963) did not find that the doubling of the length of the intervals results in appreciable decrease of final swimming speeds of coho salmon. Thus, 10-min intervals may prove entirely justifiable when the velocity increments are as small as ours.

High concentrations of free carbon dioxide had a more pronounced effect on the swimming speed of coho salmon at dissolved oxygen concentrations near or above the air-saturation value than at oxygen concentrations far below air-saturation (Fig. 6). This observed difference in effectiveness of carbon dioxide at different dissolved oxygen levels will be considered in the light of available information concerning the influence of the gas on the affinity of fish blood for oxygen.

A notable characteristic of fish blood is its very incomplete oxygenation even at extremely high oxygen tensions in the presence of moderate amounts or tensions of carbon dioxide. The large effect of carbon dioxide on the affinity of blood for oxygen at high oxygen tensions was first noted in several species of marine fish by Root (1931), and it is often called the Root effect, though it may be regarded by some as inseparable from the well-known Bohr effect. The Root effect has been observed in salmonid fishes (Ferguson and Black, 1941; Black et al., 1966a, b, c, d).

With increasing activity of a fish, the oxygen requirement of its tissues increases. The intensity of sustained activity therefore may, under some conditions, be limited by the ability of the blood to transport oxygen to the tissues. It can also be limited by the rate of delivery of oxygen to the blood at the gills when this rate is insufficient for full utilization of the oxygen transport capacity of the blood. There may be other limiting factors.

We do not yet know whether the final swimming speed of healthy (not anemic) salmon at normal carbon dioxide concentrations and high dissolved oxygen levels at which the speed is virtually independent of oxygen concentration is or is not limited by the oxygen transport capacity of their blood. Davis et al. (1963) and Brett (1964) have discussed the factors that may limit swimming speeds and oxygen uptake rates of salmon. They have arrived at no definite conclusion that can be regarded as an answer to the above question. Basu (1959) perhaps unjustifiably equated the maximum oxygen uptake rate of active fish in well-oxygenated water to "the ability of the fish to transport oxygen from the external medium under activity". He found no direct relation between the effects of carbon dioxide on the "active" oxygen uptake rates of various fish at high oxygen concentrations and its effects on the ability of their bloods to take up oxygen. Nevertheless, it seems reasonable to suppose that the level of activity that coho salmon can maintain at high oxygen concentrations in the ambient medium may be reduced by carbon dioxide at least in part through reduction of the ability of the blood to transport oxygen.

As the concentration of oxygen decreases from a high level in water whose carbon dioxide content is not too high, extraction by active fish of sufficient oxygen from the water may be maintained by passing enough water over the gills. However, after a point has been reached where the greatest possible irrigation of the gill surfaces can barely supply enough oxygen for nearly complete oxygenation of the blood, the level of sustainable activity must decrease with further reduction of dissolved oxygen. At low oxygen concentrations, the maximum sustainable activity is believed to be mostly or entirely

determined by the maximum possible rate of gill irrigation; the blood's largely unutilized oxygen transport capacity would not be truly limiting, though it may have some indirect import (Davis et al., 1963). Therefore, it appears that the effect of moderately high carbon dioxide concentrations on the ability of the blood to transport oxygen, which effect may be very important at high oxygen concentrations, should be less important or of no consequence, in relation to swimming performance, at low oxygen concentrations. At a low oxygen concentration, the extent of oxygenation of the blood at the gills is a function of the amount of oxygen delivered to the gills, and the amounts of oxygen taken up at the gills by bloods having normal and reduced affinities for oxygen therefore need not necessarily differ materially.

It must be admitted that the above explanation of our finding can be reasonably criticized as an extreme oversimplification of a complex problem; when it is pressed, some serious weaknesses or difficulties become apparent. Oxygen moves at the gills from the ambient medium into the blood plasma and thence into erythrocytes at varying rates dependent on diffusion gradients maintained by gill irrigation and tissue respiration. The oxygen content of blood returning to the gills depends on the degree of discharge of oxygen from the blood to the respiring tissues, also by diffusion. The rates of both the loading of the blood with oxygen and its unloading must be influenced by carbon dioxide. Equilibria like those on which oxygen dissociation curves for blood are based probably are never directly involved in the operation of the complex and dynamic system under consideration here. It is evident that only at very high oxygen tensions of the external medium is nearly full utilization possible of the oxygen capacity of the blood of a very active coho salmon when little carbon dioxide is present. Much lower tensions doubtless would suffice for full oxygenation of the blood's hemoglobin upon attainment of equilibrium.

Still, the reduction by carbon dioxide of the affinity of the bloods of salmonids for oxygen that has been reported must result in reduction of the degree of oxygenation of these bloods when the fish are swimming rapidly in water with very high oxygen tensions. Also, the fact remains that the maximum swimming speeds of our coho salmon at low and high carbon dioxide concentrations were virtually equal when the dissolved oxygen level was very low. We can conclude that there was no material effect of carbon dioxide on the amount of oxygen that could be extracted by the fish from the oxygen-deficient water passing over their gills. Thus, it appears that the proposed explanation of our results, or of the convergence of the curves in Fig. 6 on the left side, may be essentially sound.

An alternative, tentative explanation of our finding could be based on the supposition that carbon dioxide somehow depresses internal (tissue) respiration of active coho salmon and their muscular power more directly than through its influence on the oxygen-carrying ability of their blood, and independently of that influence. Such an effect also could well become less important and finally disappear under hypoxial conditions as the oxygenation

of the blood at the gills and the supply of oxygen to the tissues are reduced. Possible inhibition of activities of some enzymes essential to maintenance of muscular function should be relatively ineffective when the oxygen supply to muscle and other tissues is highly deficient and severely restricts tissue respiration. The importance of the effect of carbon dioxide on blood and oxygen transport perhaps has been overemphasized in the past.

Our incidental observation that a large increase of free carbon dioxide from about 50 (39–60) mg/liter to about 100 (95–120) mg/liter had no apparent effect on the swimming performance of coho salmon at rather high oxygen concentrations (Fig. 5) is interesting. Such increases of carbon dioxide concentration have a much greater effect on dissolved oxygen lethal thresholds (asphyxial levels) for coho salmon than do increases of free carbon dioxide from low levels to 50 mg/liter (McNeil, MS, 1956). However, Root (1931) found that increases of carbon dioxide beyond certain levels had little or no effect on the affinity for oxygen of the bloods of some marine fishes, and the same has been found to be true of the blood of a salmonid fish (Black et al., 1966d). The noted behavior of blood in vitro could help to explain our concordant finding pertaining to the swimming speeds of coho salmon, if that finding can be verified. The agreement of the two kinds of data could then be cited as evidence supporting the supposition that the swimming speed of coho salmon in our tests at high concentrations of both oxygen and carbon dioxide was limited by the ability of the blood to transport oxygen.

Basu (1959) found that the logarithms of the "active" oxygen consumption rates of fish at all oxygen concentrations generally decrease linearly with increase of free carbon dioxide concentration from zero. Carbon dioxide usually had about as much effect on these rates at moderately reduced oxygen concentrations as at high concentrations. Its effect on the "active" oxygen uptake rates of three tolerant species of warmwater fish was considerably greater at very low concentrations than at higher concentrations.[7] The apparent disagreement of Basu's findings with our observation that carbon dioxide had little or no effect on the swimming speed of coho salmon at low oxygen concentrations is noteworthy and puzzling. The maximum sustained swimming speed of fish and their maximum oxygen uptake rate at any given temperature are generally assumed to be closely related (Fry, 1957). The lack of any demonstrable impairment of the swimming performance of our largemouth bass by rather high carbon dioxide concentrations at any dissolved oxygen level

[7]The lowest level of dissolved oxygen at which tests with brook trout, *Salvelinus fontinalis*, the only salmonid tested by Basu (1959), were performed was about 5 mg/liter (44% of air-saturation at the test temperature of 10 C), and no marked difference was observed in effects of carbon dioxide at this and higher oxygen concentrations. At the lowest oxygen concentration tested, the trout lost its ability to be active at a carbon dioxide level somewhat above 50 mg/liter, but maintained a nearly constant oxygen uptake rate with increase of free carbon dioxide up to 80 mg/liter. Some of Basu's "active" oxygen uptake rates for warmwater fish tested at very low oxygen concentrations or at high carbon dioxide concentrations appear to be much less than "standard" or "minimum resting" rates for the same fish species under more favorable conditions. It is not clearly stated that the fish in question were not actually swimming steadily, but they probably were incapacitated and inactive.

(Fig. 3) is interesting in view of the pronounced influence of low concentrations of carbon dioxide on the oxygen uptake rates of other warmwater species reported by Basu (1959).

Our evidence that the effect of free carbon dioxide on the swimming performance of coho salmon decreases markedly with acclimation (Fig. 6) is not deemed conclusive. However, it is in agreement with the findings of other investigators, who noted that the effects of moderate (tolerable) increases of carbon dioxide concentration on the ability of fish to extract oxygen from their medium are largely or entirely transitory (McNeil, MS, 1956; Saunders, 1962; Doudoroff and Warren, 1965). And even our relatively unacclimated (least acclimated) coho salmon had been exposed to the elevated carbon dioxide concentration (about 18 mg/liter) for some time before their swimming performance was measured; without such acclimation, the performance perhaps would have been impaired much more than it was.

In the presence of much dissolved oxygen, the final swimming speeds of largemouth bass at 25 C were generally less than those of coho salmon of about the same size at 20 C. However, had a single vertical scale been used in plotting the data for largemouth bass and coho salmon in Fig. 4, the comparable curves for the two species would have met or intersected, showing the bass to be better swimmers than the coho salmon at dissolved oxygen concentrations below 2 mg/liter. Figure 4 shows that the median final swimming speed of coho salmon (i.e. the final speed of third-failing fish) at the dissolved oxygen concentration of 3 mg/liter is less than the corresponding speed at the air-saturation level of dissolved oxygen by about 30%, whereas the speed of largemouth bass at the 3 mg/liter level is less than the speed at the air-saturation level of dissolved oxygen by only about 10%. Reduction of the sustained swimming speed of largemouth bass (median final speed) by 30%, from the speed at the air-saturation level of dissolved oxygen, can be seen to occur at an oxygen concentration near 1.5 mg/liter. The curves based on mean final swimming speeds in Fig. 2 and 5 differ very little from the corresponding curves in Fig. 4 based on median final speeds.

It is interesting to note that the growth rate of largemouth bass kept on unrestricted food rations at 26 C and low carbon dioxide levels decreases materially with decrease of oxygen concentration from the air-saturation level to 5 or 6 mg/liter (Stewart et al., 1967), whereas the sustained swimming speed of our bass (Fig. 2) did not. This observation may be viewed as an indication that the swimming speed of the bass at high oxygen concentrations was not limited by the oxygen transport capacity of their blood (which seemingly could not be fully utilized at the reduced oxygen concentrations in the experiments on growth) and the supply of oxygen to their tissues. However, its significance is uncertain, for the oxygenation of blood at the gills may be more rapid in very rapidly swimming bass than in heavily fed and relatively quiet bass. The accelerated oxygenation of blood could make possible nearly full utilization of the blood's transport capacity at relatively low dissolved oxygen levels in the active bass consuming oxygen at a maximal rate. Saunders (1962)

has suggested that rapid swimming may even interfere with the respiration of fish, reducing the respiratory volume, but no conclusive evidence was presented.

The sustained swimming speed of coho salmon was not much affected at considerably reduced oxygen concentrations by 18 mg/liter of carbon dioxide after some acclimation (Fig. 6); that of somewhat acclimated largemouth bass was not adversely affected even by 48 mg/liter of carbon dioxide (Fig. 3). Ellis (1937) determined free carbon dioxide concentrations in various American rivers and found that they generally did not exceed 14 mg/liter in polluted as well as unpolluted river waters. Alabaster et al. (1957) have stated that carbon dioxide concentrations up to 50 ppm have been observed in a stream containing sewage effluent. The dissolved oxygen content of the water was not stated. Stream waters polluted with mineral acids conceivably could have temporarily even much higher concentrations of liberated carbon dioxide. However, in natural and polluted waters that are not seriously deficient in dissolved oxygen, concentrations of free carbon dioxide certainly do not often approach even 20 mg/liter. The results of our experiments thus do not indicate that free carbon dioxide can often materially impair the swimming performance of freshwater fish in nature. It should be noted, however, that greater effects of carbon dioxide on swimming performance perhaps would have been found had the test temperature been much lower. Coho salmon in our experiments were tested at a temperature (20 C) that is rather high for salmon. Basu (1959) found that the effect of carbon dioxide on active oxygen consumption rates of various fishes, including brook trout, was somewhat less at high temperatures than at lower temperatures.

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Fluorimetric Method for the Determination of Cortisol in Small Quantities of Salmonid Plasma[1]

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ABSTRACT

A method is described for the fluorimetric determination of cortisol in 1-ml aliquots of salmonid plasma. Validation experiments are presented which indicate that the reliability of the method is satisfactory. The method provides the means for the determination of low cortisol concentrations, such as those encountered in hypophysectomized or resting salmonids (0.01–0.05 μg/ml).

RÉSUMÉ

Une méthode est décrite pour la détermination du cortisol dans les aliquotes de 1 ml de plasma de salmonidés. Des expériences sur la validité de la méthode sont présentées et indiquent qu'elle a un degré d'exactitude satisfaisant. La méthode permet la détermination de faibles concentrations de cortisol telles qu'on en rencontre chez les salmonidés qui ont subi l'hypophysectomie et ceux à l'état de repos (0.01–0.05 μg/ml).

INTRODUCTION

In the past various methods have been used for the determination of cortisol in salmonid plasma; these include the phenylhydrazine, ultraviolet, and fluorescence techniques (Idler et al., 1959; Schmidt and Idler, 1962; Idler et al., 1964; Leloup-Hatey, 1964; Phillips et al., 1959). Of these methods only the fluorescence technique is sufficiently sensitive to measure cortisol in small volumes of plasma. It has been demonstrated by Frankel et al. (1967) that extraction methods and fluorimetric techniques developed for the determination of corticosteroids in a certain class of vertebrates cannot be applied to other vertebrate classes without adequate validation experiments and the incorporation of necessary modifications. In this laboratory the fluorimetric technique used for determination of cortisol in mammalian plasma by Van der Vies (1961) was found to give unreliable results when applied to the determination of cortisol in small volumes of salmonid plasma. This paper describes a method, which has evolved in this laboratory, that gives satisfactory results when applied to the determination of cortisol in 1-ml aliquots of salmon and trout plasma.

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MATERIALS AND METHODS

Reagents

The methylene chloride, carbon tetrachloride, and ethyl alcohol solvents were reagent grade and were redistilled through a Vigreux column prior to use, the first and last fractions being discarded. The carbon tetrachloride was further purified by shaking 800 ml of the solvent with 150 ml of distilled water and 50 ml of ethyl alcohol. Fluorimetric grade sulphuric acid (Fisher Scientific) was boiled for 3 hr and stored in the refrigerator (Purves and Sirett, 1965). Fluorescence reagent was prepared each week by adding three volumes of sulphuric acid very slowly to one volume of ethyl alcohol. During the addition of the sulphuric acid the flask containing the ethyl alcohol was swirled in a cooling bath containing crushed ice and water. The fluorescence reagent was kept in the refrigerator and was used while cold. The standard stock solution of cortisol (Merck) contained 1000 μg/ml of ethanol and was used to make a working standard of 1.0 μg/100 ml distilled water. The [4-^{14}C] cortisol (New England Nuclear Corp.) had a specific activity of 45 mc/mmole. Scintillation fluid was prepared which contained 4.0 g of 2,5-diphenyloxazole (PPO) and 50 mg of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP).

Equipment

A Baird-Atomic, Inc. Fluorispec model SF-1 fluorescence spectrophotometer was used for fluorimetric measurements. The excitation wavelength was 475 m, and light emitted at 525 m was monitored. The cuvettes were quartz 11 mm ID and required 1 ml of solution. Extraction tubes were conical glass-stoppered centrifuge tubes of 10-, 12-, and 15-ml capacity. After use these tubes were washed with detergent and water, rinsed in distilled water, and then boiled in a mixture of equal parts of concentrated nitric acid and water. The tubes were rinsed five times in distilled water after the acid wash and then placed upside down in a drying oven. A Packard Tri-Carb Liquid Scintillation Spectrometer was used for ^{14}C determinations.

Fish

The blood used in these experiments was peripheral blood collected from either rainbow trout (*Salmo gairdneri*) obtained from Sun Valley Trout Farm, B.C., or sockeye salmon (*Oncorhynchus nerka*) caught during the freshwater stage of their spawning migration and held in aquaria at this laboratory. The method has also been used for the determination of cortisol in plasma of coho salmon (*O. kisutch*) and spring salmon (*O. tschawytscha*).

Extraction Procedure

Immediately after collection, heparinized peripheral blood was centrifuged at 2 C and 3000 *g* for 10 min. The plasma was either extracted immediately after centrifugation or stored at –30 C. A 1.0-ml aliquot of plasma was pipetted into a 15-ml conical centrifuge tube and diluted to 2.0 ml with distilled water.

After the addition of 12 ml of methylene chloride from an automatic syringe, the centrifuge tube was stoppered using a glass stopper wetted with distilled water. The tube was then shaken for 30 sec, and centrifuged to separate the aqueous and methylene chloride phases. The aqueous layer was removed by suction using a Pasteur pipette connected via a trap to a vacuum line. A 10-ml aliquot of the methylene chloride layer was transferred with a 10-ml syringe and long needle to a 12-ml graduated centrifuge tube. Ethanol (0.5 ml) was added to the methylene chloride and the solution was then evaporated under a stream of nitrogen in a water bath maintained at 45 C. The residue was dissolved in 2.0 ml of distilled water and shaken with 10 ml of carbon tetrachloride. After centrifugation the carbon tetrachloride was removed by a syringe with a long needle and discarded. The aqueous layer was reextracted with a further 10 ml of carbon tetrachloride. The cortisol was now extracted from the aqueous layer by shaking with 10 ml of methylene chloride. After centrifugation an 8.5-ml aliquot of the methylene chloride phase was transferred to a 10-ml graduated centrifuge tube. A 1.5-ml aliquot of fluorescence reagent was now added from a pipette and the tube was stoppered with a dry stopper and shaken for 30 sec. After centrifugation the methylene chloride was removed by suction and the reagent layer was poured into an 11-mm ID cuvette. Two standards consisting of 0.2 ml of working standard (0.2 μg cortisol) diluted to 2 ml with distilled water and two blanks consisting of 2 ml of distilled water were also carried through the above procedure at the same time as the plasma. The spectrophoto-fluorimeter was set to give a scale reading of 90 with one of the cortisol standards. The other standard, blanks, and unknowns were then read relative to the first standard (Scheme I).

Statistical treatment.— Analytical results were expressed as mean $\pm$ one standard deviation of the mean.

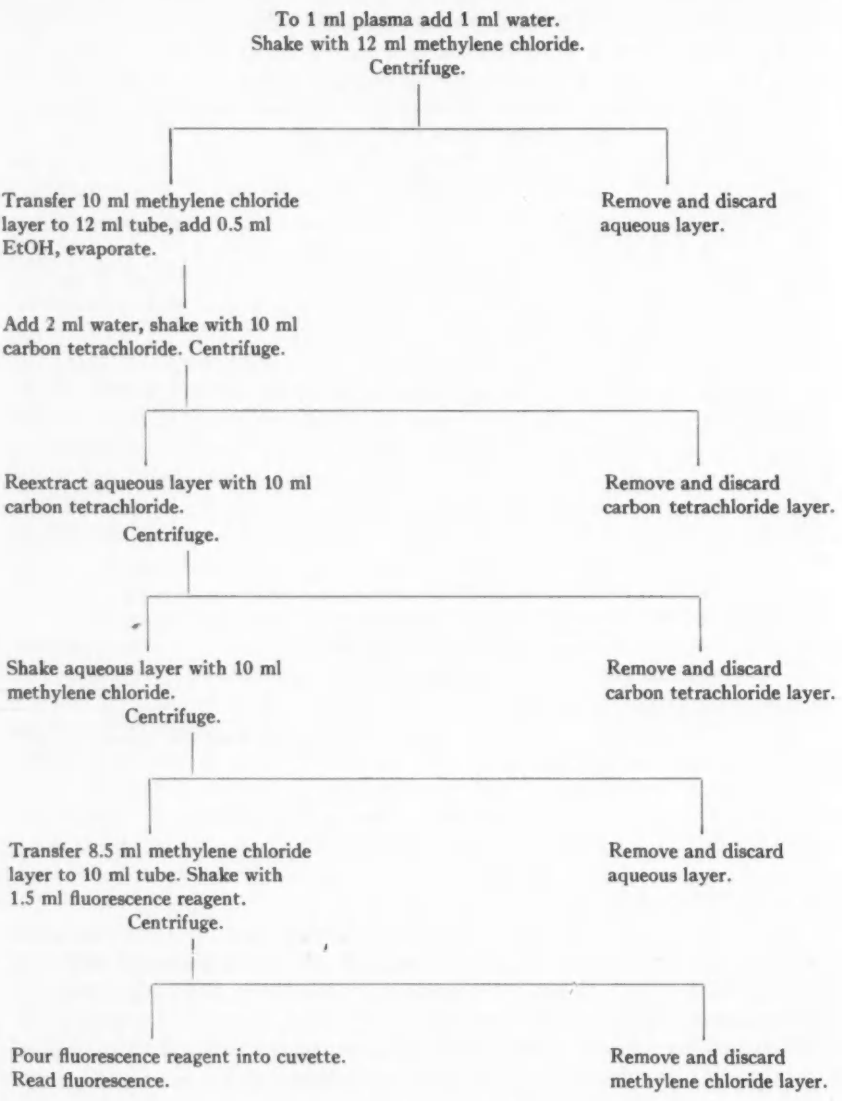
RESULTS

Development of Fluorescence

The most suitable time for reading the fluorescence due to cortisol was determined by carrying both cortisol standards and trout plasma of different cortisol concentrations through the extraction procedure and then observing the rate of development of fluorescence in each case (Fig. 1). The major part of the fluorescence in the standard and plasma cortisol extracts developed in 30 min and we decided to read all samples at this time.

Volume of Plasma

Moncloa et al. (1959) on the basis of results from their assay for corticosterone drew the conclusion that fluorescence was a linear function of the amount of plasma used for the assay and that the line had a positive intercept with the ordinate. We have investigated this possibility in our assay for cortisol. In four experiments carried out, the relationship between plasma volume and


To 1 ml plasma add 1 ml water.
Shake with 12 ml methylene chloride.
Centrifuge.
Transfer 10 ml methylene chloride layer to 12 ml tube, add 0.5 ml EtOH, evaporate.
Remove and discard aqueous layer.
Add 2 ml water, shake with 10 ml carbon tetrachloride. Centrifuge.
Reextract aqueous layer with 10 ml carbon tetrachloride.
Centrifuge.
Remove and discard carbon tetrachloride layer.
Shake aqueous layer with 10 ml methylene chloride.
Centrifuge.
Remove and discard carbon tetrachloride layer.
Transfer 8.5 ml methylene chloride layer to 10 ml tube. Shake with 1.5 ml fluorescence reagent.
Centrifuge.
Remove and discard aqueous layer.
Pour fluorescence reagent into cuvette.
Read fluorescence.
Remove and discard methylene chloride layer.

SCHEME I. Outline of extraction procedure.

fluorescence reading appeared to be slightly curvilinear with the extrapolated line passing through the origin (Fig. 2). However, deviation from linearity was not statistically significant in any of these experiments (Snedecor, 1946).

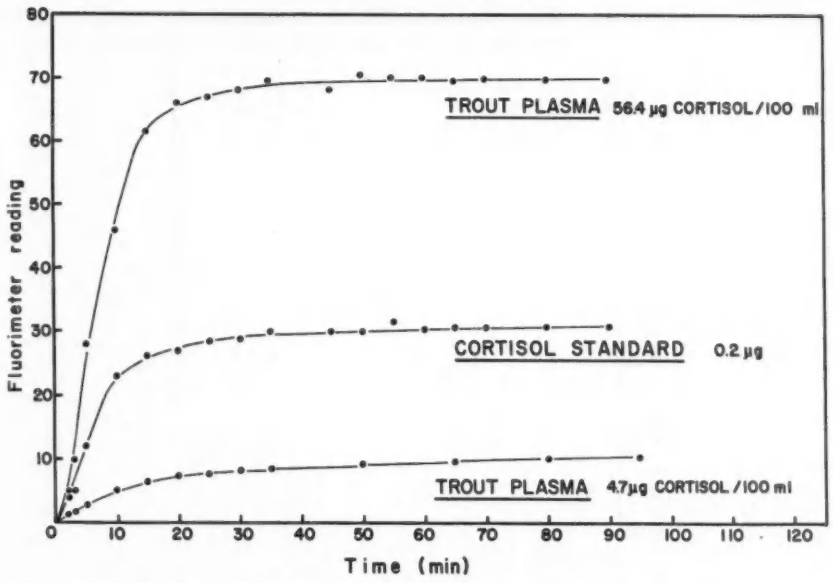


FIG. 1. The influence of time on the development of fluorescence of cortisol and trout plasma carried through the extraction procedure.

It was evident from these determinations on different volumes of plasma that the ratio of standard deviation to machine reading was similar for volumes of 1 ml and 2 ml of plasma. However, when 0.5 ml of plasma was used the standard deviation increased in relation to the machine reading. Thus 1.0 ml of plasma is the minimum volume which can be used without loss of precision.

RECOVERY OF CORTISOL

The recovery of cortisol and its reproducibility were determined by carrying samples containing [4-^{14}C] cortisol through the extraction procedure. [4-^{14}C] cortisol (4660 count/min) was added to each of 10 aliquots of a plasma pool and also to three cortisol standards and three blanks. The samples were carried through the extraction procedure as far as the final extraction with methylene chloride. At this point, in addition to removing 8.5 ml of the extract for determination of cortisol by fluorescence, a 0.5-ml aliquot of the methylene chloride was placed in a glass scintillation counter vial and evaporated under nitrogen. After addition of 10 ml of scintillation fluid the radioactivity was determined in a scintillation counter. The recovery of radioactivity from the plasma samples when corrected for volume losses was 75.4 $\pm$ 1.8%. The recovery of radioactivity from the standards and blanks carried through the procedure was 80.3 $\pm$ 2.0%. The cortisol concentration in this plasma pool

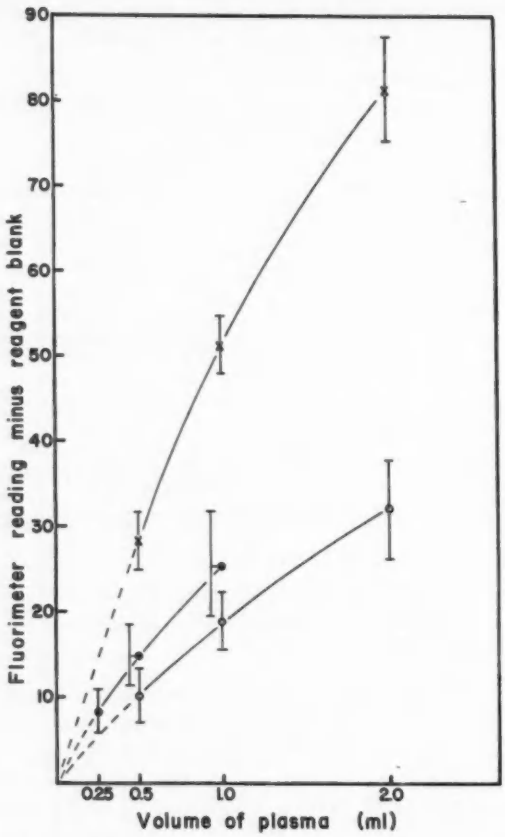


FIG. 2. The effect of plasma volume on fluorescence reading in three separate pools of trout plasma. × —— 18.0 μg cortisol/100 ml; ● —— 6.2 μg cortisol/100 ml; ○ —— 4.7μg cortisol/100 ml. Vertical bars are standard deviations.

was 5.6 ± 0.9 μg/100 ml. Since the standard deviation of the fluorescence reading was considerably greater than the standard deviation of the recovery of radioactive cortisol it appears that the extraction of cortisol from the plasma is more reproducible than the extraction of fluorogenic material from the plasma and the subsequent development and measurement of its fluorescence.

Recovery was also determined by adding 0.05, 0.10, 0.20, and 0.40 μg of cortisol to aliquots of a pool of salmon plasma. There were six samples of plasma at each concentration and they were carried through the fluorimetric procedure together with six 0.20-μg cortisol standards and two blanks. The

observed cortisol concentrations fell on a line which had slightly less slope than the theoretical line (Fig. 3).

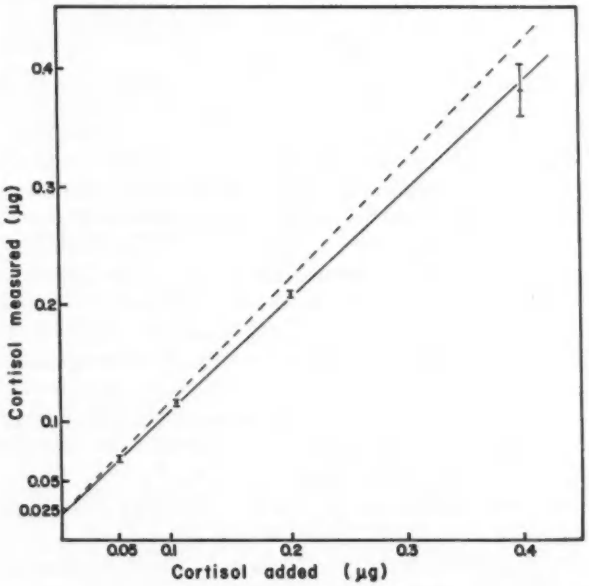


FIG. 3. Cortisol determination after the addition of known amounts of cortisol to 1.0-ml aliquots of a pool of salmon plasma: broken line, theoretical line; solid line, observed line. Vertical bars are standard deviations.

PRECISION AND SENSITIVITY

Two plasma pools were prepared and the cortisol concentrations were determined in 10 samples from one pool and 12 samples from the second pool. The cortisol concentrations were 5.6 ± 0.9 and 4.2 ± 0.8 µg/100 ml respectively. The precision of duplicate determinations was determined using the formula of Snedecor (1952):

$$s = \sqrt{\frac{d^2}{2N}}$$

in which d is the difference between duplicate determinations and N is the number of duplicate determinations. For concentrations between 0 and 10 µg/100 ml, s equaled 0.5 µg (N = 47).

The sensitivity or minimum concentration which differs significantly from zero was calculated using the following equation:

$$\text{sensitivity} = \frac{t\,s}{\sqrt{N}}$$

t being Student's t value ($p = .01$), s the standard deviation, and N the number of determinations. The sensitivity for a single determination was 0.7 μg/100 ml plasma (N = 12).

DISCUSSION

Frankel et al. (1967) in an extensive study of the determination of corticosterone in avian plasma using fluorimetric techniques have shown that techniques applicable to one vertebrate for the fluorimetric determination of corticosteroids are not necessarily applicable to other vertebrates owing to the presence of interfering fluorogens in the plasma. In the present method the petroleum ether wash used by Van der Vies (1961) was omitted. Vermeulen and Van der Straeten (1964) working with human plasma also found this step to be unnecessary. In addition it was found that omitting the sodium hydroxide wash used by the above workers resulted in greater reproducibility. In the method described here the time allowed for the development of fluorescence was chosen so as to allow a maximum contribution of cortisol fluorescence while minimizing fluorescence caused by non-specific fluorogens. In the first method to be developed which used a carbon tetrachloride extraction (Van der Vies, 1961) the development time was 1 hr. In order to minimize non-specific fluorescence Vermeulen and Van der Straeten (1964) chose a development time of 10 min. It is evident from our results that at 10 min the cortisol fluorescence is still increasing rapidly reaching a plateau at 30 min. Thus fluorimetric determinations were made at 30 min.

The experiments in which cortisol was determined in various volumes of plasma indicated that there was very little residual fluorescence at zero volume indicating high specificity (Moncloa et al., 1959; Vermeulen and Van der Straeten, 1964). The 75.4 ± 1.8% recovery of [4-^{14}C] cortisol added to plasma samples and carried through the extraction procedure indicates the high level of precision obtained with this extraction procedure. The precision determined from the difference between duplicate determinations agrees favourably with the values obtained by Vermeulen and Van der Straeten (1964). The sensitivity of 0.7 μg/100 ml for a single determination obtained in the present method is an improvement over that obtained for duplicate determinations by the above authors.

The method has proved to be reliable over the wide range of cortisol concentrations found in salmonid plasma and, as only 1.0 ml of plasma is required per determination, it is possible to take serial blood samples from a large fish and investigate the time course of changes in cortisol concentration (Fagerlund, 1967). The determination of corticosteroid levels in hypophysectomized animals and animals in which the release of adrenocorticotrophin has been blocked by the injection of dexamethasone provides a sensitive method for determining the presence of interfering fluorescent material (Frankel et al., 1967). We have carried out such determinations on *Salmo gairdneri* and have shown the cortisol concentrations in hypophysectomized

and dexamethasone injected trout (Donaldson and McBride, 1967) and resting salmon (Fagerlund, 1967) to be very low, indicating an absence of significant amounts of interfering fluorescent material.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Mrs Helen Dye.

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Autumnal and Over-Winter Limnology of Three Small Eutrophic Lakes with Particular Reference to Experimental Circulation and Trout Mortality[1,2,3]

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ABSTRACT

The autumnal and over-winter limnological characteristics of two small eutrophic lakes differed considerably from those of a third eutrophic lake; all were close to each other in the southwest interior of British Columbia. Thermal and chemical stratification was well defined in Marquette and Corbett lakes because local topography provided protection from wind action; stratification in the more exposed Courtney Lake was ill defined. Average wind velocities during summer and autumn on Courtney Lake were 4.3 times as great as, and more unidirectional than, those on Corbett Lake and probably Marquette. Complete natural autumnal oxygenation and circulation in Courtney Lake provided comparatively high concentrations of dissolved oxygen which permitted the over-winter survival of *Salmo gairdneri* and *Richardsonius balteatus*. Although Marquette and Corbett lakes were isothermal in late autumn, oxygen concentrations were well below saturation levels and circulation was probably incomplete. Consequently oxygen depletion during winter was severe and *S. gairdneri* and *Salvelinus fontinalis* were subject to over-winter mortality.

Experimental circulation of Corbett Lake, just prior to ice cover, confirmed the hypothesis that incomplete autumnal oxygenation is a cause of "winter kill" of fishes. Artificial autumnal circulation of the lake provided an oxygen concentration sufficient to prevent over-winter fish mortality. However, over-winter mortality of the entire fish population did occur in the control lake, Marquette (*incomplete* autumnal oxygenation) but not in Courtney Lake (*complete* autumnal oxygenation).

INTRODUCTION

Shallow, eutrophic lakes that have 3–4 months of ice and snow cover are often subject to over-winter mortality of fishes (Greenbank, 1945; Welch, 1952; Hutchinson, 1957; Reid, 1961). This phenomenon is commonly attributed to severe oxygen depletion during the period that the ice cover effectively seals off the lake from atmospheric oxygen. Typically, over-winter mortality of fishes occurs in highly productive lakes that completely turn over in the autumn and are fully saturated with oxygen prior to the formation of

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an ice cover. However, this typical sequence of events was not evident in Marquette and Corbett lakes, in the southwest interior of British Columbia. Neither Marquette nor Corbett was saturated with oxygen in the autumn. Over-winter mortality of fishes occurred in Corbett Lake in 6 of 10 recent years and in Marquette Lake in 2 of 3 years. Unexpectedly, Courtney Lake, a shallower nearby lake, had no record of over-winter mortalities.

Studies of the autumn and winter limnology of Corbett and Courtney lakes were initiated in 1961 and in 1962 these were extended to include Marquette Lake. To test the hypothesis that differences in winter oxygen concentrations between the lakes were due to lack of complete autumnal oxygenation, Corbett Lake was artificially circulated in the autumn of 1962 and 1963.

Puke (1949), Schmitz and Hasler (1958), Burdick (1959), Schmitz (1959), Foster (1960), Rassmussen (MS, 1960), Patriarche (1961), Woods (1961), Carufel (1962), and others all either suggest or report on the compressed-air method used to induce artificial circulation. The method has been primarily used to remove part of the winter ice cover after it has formed, thus making atmospheric oxygen available for solution. However, the object in this study was to build up a reserve of dissolved oxygen *just before the winter ice cover formed*.

STUDY AREA

Marquette, Corbett, and Courtney lakes are situated between lat 50°N and 50°53′N and long 120°35′W and 120°40′W. The first two lakes are at an elevation of 1036 m and the last at 975 m. Topography of the surrounding area is shown in Fig. 1 and the orientation of lake basins and hydrography in Fig. 2.

Generally, the southwestern interior of British Columbia has a mild, continental climate. Most of the annual precipitation occurs as a mean annual snowfall of 101.6 cm, chiefly in December, January, and February. Directions of air currents differ considerably but valleys canalize winds, in most cases from north or south (Kendrew and Kerr, 1955).

The littoral zones of the three lakes were characterized by an abundance of *Chara* sp. No marked blooms of phytoplankton were observed during spring, summer, or autumn.

Zooplankters that commonly occur in Corbett Lake are listed by Teraguchi and Northcote (1966) and representatives of the same genera likely occur as well in Marquette and Courtney lakes. Benthic organisms found in Corbett and Courtney lakes are listed by Humphreys (MS, 1964).

Rainbow trout (*Salmo gairdneri*) and brook trout (*Salvelinus fontinalis*) were stocked annually in Marquette and Corbett lakes in about equal numbers. A stocked population of rainbow trout was present in Courtney Lake along with substantial numbers of redside shiners (*Richardsonius balteatus*).

FIG. 1. Locations of the study lakes with respect to surrounding topography (contours in meters).

METHODS

Hydrographic maps for the three lakes were drawn from soundings of a Furuno 200 kc, model F701, recording echo sounder.

Meteorological data were collected by two totalizing anemometers (Munro, London) and two wind direction recorders (Schmidt and Marshall, 1960). These were mounted 0.5 m above the lake surfaces on rafts on Corbett and Courtney lakes (Fig. 2). Because of lateral movement of the rafts about the aligned position (about 10–30°), only those directions shown in Fig. 4 were distinguishable on the recording charts. Consequently, wind directions on both lakes were measured for only about 63% of the recorded period, or an average of 107 and 103 hr per week on Corbett and Courtney lakes respectively. The expected weekly average shown in Fig. 4 was determined by summing the weekly total time for all wind directions and dividing by the number of directions recorded (9).

Transparency was measured in spring, summer, and autumn with a standard Secchi disk (20 cm diam) using a glass viewing box. Underwater

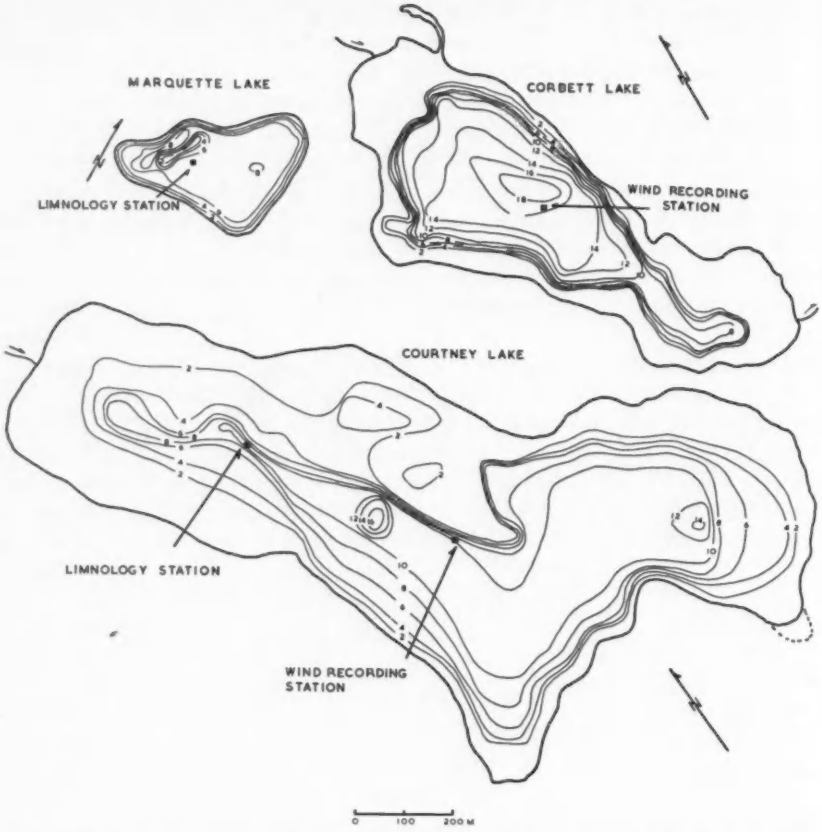

FIG. 2. Hydrographic maps of the study lakes. Depth contours in meters. Limnology station 1 is shown for Corbett Lake in Fig. 3.

light measurements were taken in winter with a photometer (Model 15-M-02/1 GM Manufacturing Co.); vertical temperature series were taken with a Telethermometer (Model 44 TB — Yellow Springs Instrument Company Inc.).

The unmodified Winkler method was used for oxygen determinations of water samples taken with a Kemmerer bottle. When taking water samples immediately below the ice, great care was taken to have the water undisturbed by ice drilling. The water samples taken from the Kemmerer bottle represented an average of the water column within the bottle (41.5 cm long). No attempt was made to make oxygen determinations on the micro stratification immediately below the ice.

Measurements of *p*H were made with two portable *p*H meters (Analytical Pocket *p*H Meter, Analytical Measurements Inc., Model N-2, Beckman). The evaporation method was used to determine total dissolved solids (American

Public Health Association Inc., 1960). These determinations were made on water samples taken periodically at the surface and bottom of each lake and the values were averaged for the year.

Phenolphthalein and methyl orange alkalinity were determined by the method outlined in American Public Health Association Inc. (1960), except that the titre was 0.10 N H_2SO_4. In situ conductivity readings were taken with a model RB_2 3341 Solu Bridge (Industrial Instruments Inc.)

Figure 3 illustrates the layout of the bubbler system, which was located in the deepest part of the lake. The circle of plastic pipe was 726 m long. The

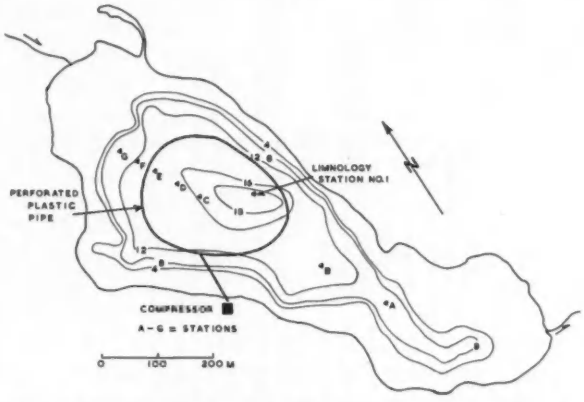

FIG. 3. Layout of artificial circulation system for Corbett Lake and locations of sampling stations.

pipe had an inside diam of 2.5 cm and 0.8-mm holes were drilled at 3.7-m intervals. An Atlas Copco V.T. 4, 4.5 m^3/min air compressor supplied air to the system. The compressor was operated at 6.3–6.7 kg/cm^2. Engineering design was done by Mr L. Pasternak of Atlas Copco of Canada Ltd., Vancouver, B.C. An attempt was made to suspend the pipe at a depth of 12–13 m from surface rafts. This was unsuccessful, however, and about 80% of the pipe lay on the bottom of the lake.

COMPARATIVE LIMNOLOGY

MORPHOMETRY

Physical differences in the three lakes are shown by a comparison of morphometric features (Table I). Characteristics that are pertinent to lake circulation and oxygenation are as follows.

Courtney Lake has the greatest area but is relatively shallow, whereas Marquette and Corbett lakes are comparatively small and deep.

The basin of Marquette Lake is U-shaped, that of Corbett Lake cone-shaped, and that of Courtney Lake saucer-shaped.

TABLE I. Morphometric features of Marquette, Corbett, and Courtney lakes.

	Marquette Lake	Corbett Lake	Courtney Lake
Area (*ha*)	5.3	24.2	71.6
Maximum depth (*m*)	9.2	19.5	17.0
Mean depth (*m*)	5.9	7.0	4.9
Maximum effective length (*m*)	141.7[a]	244.3[a]	946.0
Maximum length (*m*)	372.9	990.0	1637.4
Volume ($\times 10^4$ m^3)	31.1	168.9	351.2
Shore line development	1.2	1.6	1.5
Ratio mean depth to maximum depth	0.64	0.36	0.29
Mean slope of basin (*degrees*)	7°55′	6°9′	3°13′
Littoral development (%)	8.5	46.4	60.3

[a]Maximum effective length is equal to mean width because winds on Marquette and Corbett lakes were predominantly variable in direction.

The maximum effective length (Welch, 1948) for the three lakes is: Courtney 946 m, Corbett 244 m, and Marquette 141 m.

METEOROLOGY

Average daily wind velocity on Courtney Lake was 199 cm/sec and that on Corbett Lake 46.2 cm/sec. Visual observations and occasional measurements indicated that wind velocities on Marquette were similar to those on Corbett.

On Courtney Lake the highest weekly average direction of the wind was from the south to the southwest (Fig. 4); this direction was recorded for 51 of every 103 hr. However, on Corbett Lake the average prevailing direction was variable for 49 of every 107 hr. Wind direction on Marquette Lake appeared similar to that on Corbett Lake.

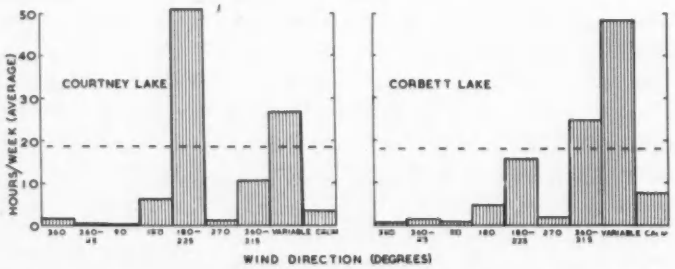

FIG. 4. Weekly average wind directions recorded on surfaces of Corbett and Courtney lakes. The broken line represents the expected weekly average, which is explained in the text.

Snow depth on the lake ice surfaces was measured at only monthly intervals (Fig. 5). However, daily records taken near the lakes by the Canada Department of Transport (1961 to 1964) showed the same annual trends as the monthly measurements.

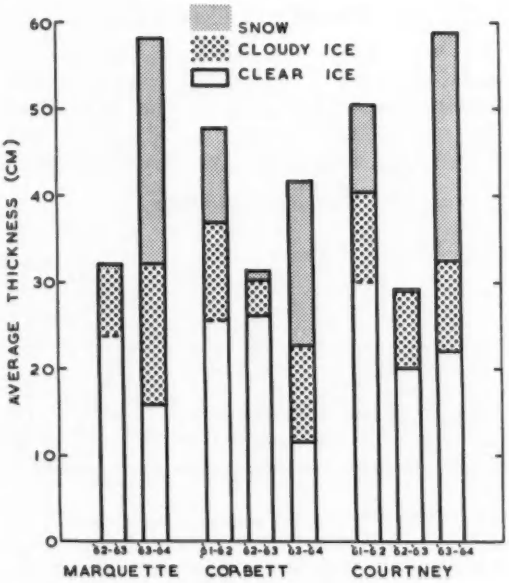

FIG. 5. Average thicknesses of ice and snow depths on the three lakes (three recordings 1962 and 1963; four recordings 1964).

SOME CHEMICAL AND PHYSICAL CHARACTERISTICS

Seasonal *p*H values ranged from a low of 7.1 in Marquette Lake to a high of 8.9 in Courtney Lake. Corbett Lake values were intermediate at 7.6–8.25. Carbonate alkalinity was detected only in Courtney Lake (Table II). Minimum bicarbonate values at the surface in January and March may have been the result of melt-water seeping through the ice.

Vertical in situ specific conductivity readings differed little between lakes at any one time. Values ranged from 40 to 200 μmhos/cm. The lowest value was recorded immediately below the ice in Courtney Lake on March 7, 1963 and the highest at the bottoms of the three lakes on August 7, 1962.

The mean concentration of dissolved solids in Courtney Lake was 406 mg/liter, in Corbett Lake 361 mg/liter, and in Marquette Lake 352 mg/liter.

Secchi disk readings, averaged for spring, summer, and autumn, were 5.5 m in Corbett Lake, 5.0 m in Courtney Lake, and 3.0 m in Marquette Lake. In winter, light intensity and penetration was less in Corbett Lake than in

TABLE II. Maximum and minimum bicarbonate and carbonate alkalinities (mg/liter) recorded in Marquette, Corbett, and Courtney lakes.

	Marquette Lake		Corbett Lake		Courtney Lake	
Bicarbonate alkalinity						
Depth (*m*):	0	7.6–8.5	0	10.7–18.3	0	8.5–10.7
Maximum:	300 (June 24/62)	365 (Mar. 4/63)	350 (Aug. 28/61)	349 (Aug. 8/62)	329 (Mar.14/62)	329 (Mar.14/62)
Minimum:	150 (Mar. 4/63)	345 (Aug. 9/62)	35 (Jan. 31/62)	310 (Sept. 30/62)	20 (Mar. 7/63)	285 (Oct. 1/62)
Carbonate alkalinity						
Maximum:	0	0	0	0	80 (May 20/62)	74 (May 20/62)
Minimum:	0	0	0	0	32 (May 13/62)	0

Courtney Lake (Fig. 6). These differences were probably due to the greater amount of snow and ice cover on Corbett Lake (Fig. 6). Differences in winter light penetration and intensity between years were considerable. In Corbett

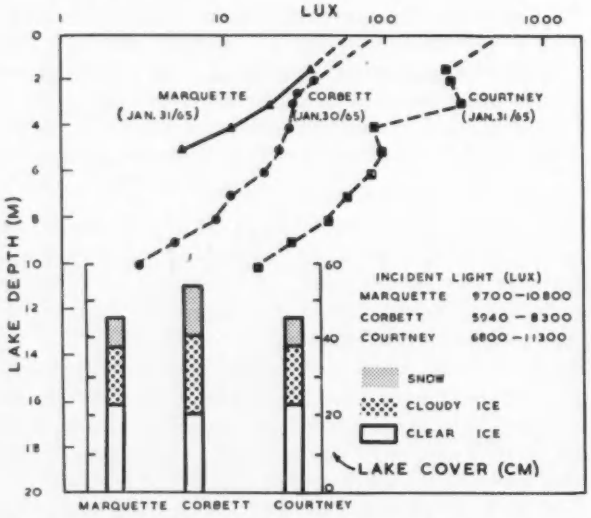

FIG. 6. Light intensity and penetration through snow and ice cover on the three lakes, January 1965. Measurements of snow and ice cover were made at the same times as those of light.

Lake an intensity of 5 lux was recorded at 15.5 m in 1963 but at only 5.25 m in 1964 (Fig. 7). Consequently, photosynthetic oxygen production would have been greatly reduced in 1964.

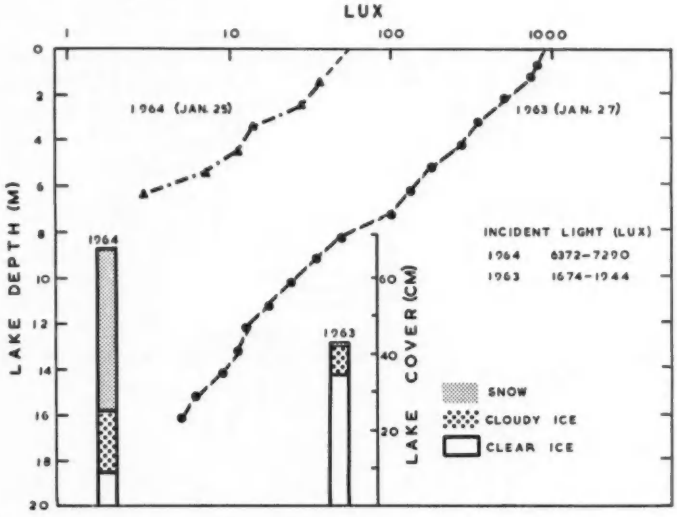

FIG. 7. Light intensity and penetration through snow and ice cover on Corbett Lake, January 1963 and 1964. Note that there is an appreciable difference in the amount of snow and ice cover between years.

TEMPERATURE AND OXYGEN STRATIFICATION

Differences in thermal and chemical stratification between Corbett and Courtney lakes were evident early in the spring and continued throughout the summer (Fig. 8, 9). The contrast in degree of stratification between lakes is well illustrated by the differences between surface and bottom temperatures in August 1962: Corbett Lake, 13.6 C; Courtney Lake, 5.6 C.

The degree of stability can be quantified in terms of the amount of work necessary to mix an entire body of water to uniform temperature without changing its mean temperature (Eckel, 1950). Schmidt's stability values (Eckel, 1950) for Corbett and Courtney lakes on July 15, 1962 were 163.4 and 52.5 gcm/cm^2 respectively. As late as the end of September there was still a great difference in stability between the lakes. By August 30 the stability value for Courtney Lake had dropped to 11.6 gcm/cm^2, whereas values of this order were not reached in Corbett Lake until October 13 (13.0 gcm/cm^2).

Similarly, in late summer and early autumn of 1961 Corbett Lake was sharply stratified, in contrast to Courtney Lake.

Inverse temperature stratification was evident each winter but Courtney Lake was warmer at the bottom than either Corbett or Marquette Lake. This

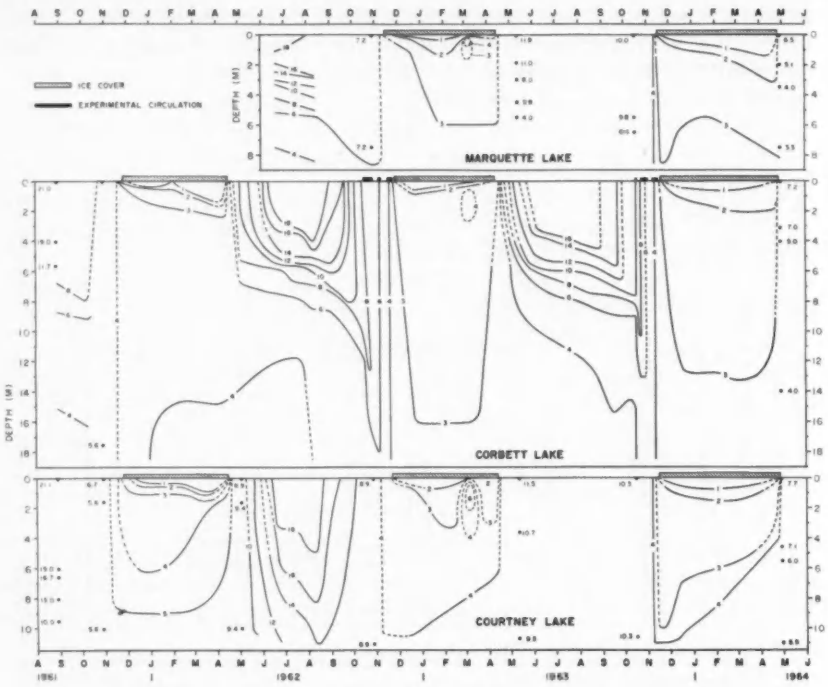

FIG. 8. Seasonal isotherms (degrees C) for the three lakes from autumn 1961 to spring 1964. Note timing of experimental circulation and duration of ice cover.

was probably due to radiant heating because of greater light penetration (Fig. 6) (Ruttner, 1963). Anomalous heating was recorded in distinct layers in March 1963 (Fig. 8). This was probably the result of heat absorbtion by a stratum of phytoplankton.

Summer oxygen concentration was generally high in Courtney Lake, lower in Corbett Lake, and lowest in Marquette Lake (Fig. 10). In summer the hypolimnion of Corbett Lake was deoxygenated but the hypolimnion of Courtney Lake was comparatively less depleted. Oxygen stratification in Marquette Lake was similar to that of Corbett Lake.

Even though Corbett Lake was homothermal in mid-autumn in 1961 (Fig. 8, October 28), the surface oxygen concentration was only 4 mg/liter whereas that in Courtney Lake was 9.5 mg/liter (Fig. 10). Thus there was a very low oxygen reserve in Corbett Lake prior to the formation of the winter ice cover. By December 28, 1961, dissolved oxygen was almost totally depleted. Dead trout of both species were observed and recovered by the use of scuba gear in January 1962 and at the same time no fish were caught in the gill nets.

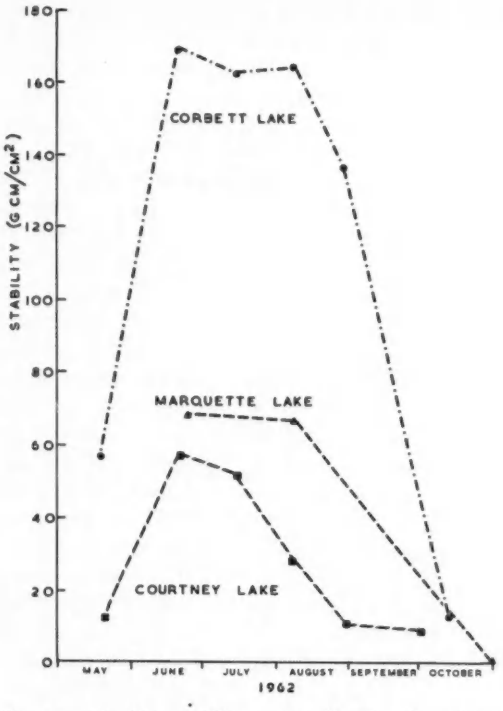

FIG. 9. Schmidt's stability values for the three lakes for the spring–autumn period of 1962.

On the other hand, Courtney Lake was well oxygenated throughout its entire water mass in the autumn of 1961. Therefore it had a comparatively high oxygen reserve prior to the formation of winter ice cover. In January and March 1962 live trout and shiners were taken in gill nets. In March, observations were made using scuba gear but neither live nor dead fish were observed.

Autumnal turnover in Marquette Lake in the autumns of 1962 and 1963 was similar to that in Corbett Lake in 1961. Although dissolved oxygen in Marquette Lake was recorded from top to bottom, concentrations were well below saturation levels immediately prior to ice cover. That no fish mortality occurred in Marquette Lake in 1962–63 was probably due to the fact that below-average snowfall allowed the formation of a narrow stratum of supersaturated oxygen of a photosynthetic origin (Fig. 10, March 1963). On the same date, trout were observed in the upper 2–3 m and fishes taken in gill nets were caught only in the upper 2–3 m of the nets. In 1963–64 with "normal" snow cover, an apparent complete mortality of fishes occurred in Marquette Lake — similar to that in Corbett Lake in 1961–62.

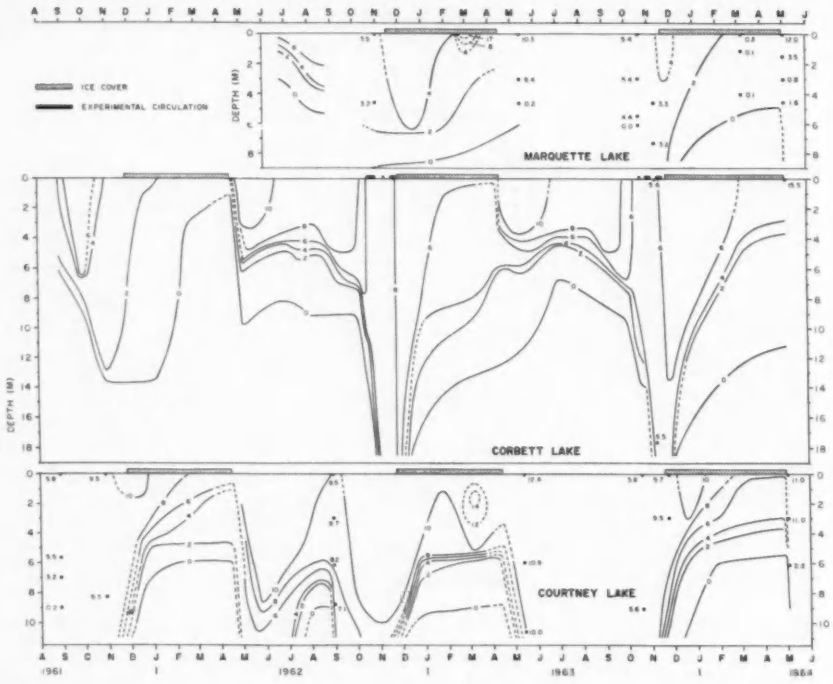

FIG. 10. Dissolved oxygen isopleths (mg/liter) for the three lakes from autumn 1961 to spring 1964.

Circulation and oxygenation were incomplete in both Marquette and Corbett lakes in the spring, as in the autumn, whereas Courtney Lake was completely circulated and oxygenated (Fig. 10).

EXPERIMENTAL CIRCULATION

When the artificial circulation system was put into operation in Corbett Lake, a column of bubbles was emitted from each hole in the perforated pipe. The column of bubbles increased in diameter as the bubbles rose to the surface. At the surface the rising bubbles created a circular "boil" about 1.5 m in diam and lifted the surface water about 10–15 cm. Lateral streaming was evident for 360° around each "boil." The action of the bubbler was similar to that described by Baines (1961). The rising bubbles lifted deoxygenated water from the hypolimnion to the epilimnion and the surface of the lake, where it absorbed atmospheric oxygen.

Artificial circulation began in 1962 at 1500 hr on October 17 and in 1963 on October 18 at 1620 hr. The immediate effects of experimental circulation were evidenced by a strong odour of hydrogen sulfide, a rapid and complete

breakdown of temperature stratification (Fig. 8), and a reduction in mean oxygen concentration (Fig. 11). The lake was isothermal to 10 m just prior to artificial circulation but quickly became totally isothermal. In 1962 Corbett Lake was isothermal at a lower temperature and at an earlier date than was Courtney Lake (Fig. 8). Initially the mean oxygen concentration was reduced from 6.30 mg/liter to 4.70 mg/liter. Subsequent artificial circulation and some natural circulation gradually increased the mean concentration to 8 mg/liter and extended the vertical penetration of dissolved oxygen to the bottom of the lake (Fig. 10, 1962). During the initial period of artificial circulation the greatest increase in mean concentration of oxygen was concomitant with the least increase in vertical penetration (Fig. 11, 1962). During the 1962 experiment the rate of increase in mean oxygen concentration was greatest during the second period of artificial circulation (Fig. 11).

Differences between natural and artificial autumnal circulation and oxygenation in Corbett Lake were marked. Autumnal oxygen isopleths for 1961 displayed slighter depressions and lower concentrations than those of either 1962 or 1963 (Fig. 10). The 0 mg/liter isopleth was recorded for the duration of natural autumnal circulation but was quickly eliminated during artificial circulation. Natural autumnal circulation in Marquette Lake during 1962 and 1963 produced low oxygen concentrations comparable with those in Corbett Lake in 1961 whereas artificial circulation in Corbett Lake produced relatively high oxygen concentrations similar to those associated with natural circulation in Courtney Lake (Fig. 10). The artificial circulation of Corbett Lake in 1962 and 1963 effectively prevented any over-winter fish mortality during the winters of 1962–63 and 1963–64.

DISCUSSION

Wind Action and Stability

Local topography shields Marquette and Corbett lakes from wind action but Courtney Lake is well exposed (Fig. 1).

Largely because of the differences in wind action throughout the summer and autumn, Courtney Lake differed markedly in chemical stratification and stability from Marquette and Corbett lakes. For the same reason, the summer temperature of Courtney Lake (Fig. 8) was higher than that of Corbett Lake.

This indicated that more work was done by the wind in stirring heat into Courtney Lake (Birge, 1916). Courtney Lake would therefore require less work to mix in the autumn because of the near-isothermal condition. The differences in the amount of work necessary to circulate the lakes are best illustrated by Schmidt's stability values (Fig. 9). About the end of June the value for Corbett Lake was nearly three times that for Courtney Lake and towards the end of the summer stagnation period it was much greater. Therefore, even if both lakes received equal amounts of wind action, its effect would have been greatest on Courtney Lake and much less on Marquette and Corbett lakes.

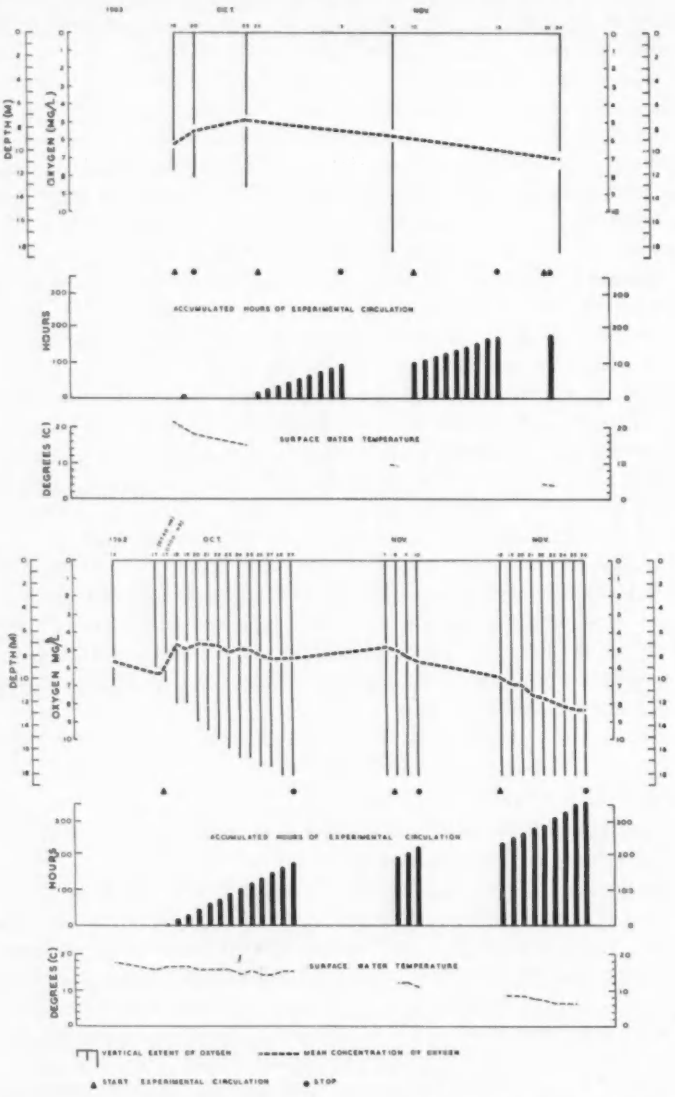

FIG. 11. Changes in mean oxygen concentrations and in vertical extent of measurable dissolved oxygen effected by the artificial circulation of Corbett Lake.

An excellent example of the differences in exposure of the lakes was provided by the effects of an above-average wind on August 25, 1962. The wind completely mixed Courtney Lake (Fig. 10, August 1962) whereas Corbett

Lake was relatively undisturbed. Although no data are available, it is likely that Marquette Lake was not mixed either. The drop in stability of Courtney and Corbett lakes between August 8 and August 31 (Fig. 9) was doubtless due to the wind of August 25. Significantly, the drop in stability was much greater in Courtney Lake (57%), than in Corbett Lake (16%).

Oxygenation

The most favorable condition for oxygenation is a highly disturbed lake surface (Hutchinson, 1957). Laboratory studies have shown that molecular diffusion and convective streaming in quiescent waters are extremely slow and alone probably play an insignificant role in the oxygenation of any sizeable water mass (Hutchinson, 1957). Determinations of gas exchange rates in natural waters revealed a low exchange coefficient when the water was stable enough to allow a gradient of dissolved oxygen at the surface.

Breaking waves were rarely observed on Corbett Lake, never on Marquette Lake but frequently on Courtney Lake. Strong prevailing winds on Courtney Lake produced sufficient surface agitation to result in effective vernal and autumnal oxygenation. On Corbett and Marquette lakes, much less wind produced little surface agitation and the minimal amount of oxygenation may have been the result of some surface agitation and convective streaming.

Experimental Circulation

Experimental circulation in Corbett Lake induced depressions in the thermocline by lifting cold water from the hypolimnion, and the rapid formation of an isothermal condition. These changes were similar to those reported by Schmitz and Hasler (1958) and Gilson (1962). Hooper et al. (1952) reported similar results using a water pump to induce circulation. Patriarche (1961) illustrated depressed isotherms in mid-winter which were the result of lifting warmer water (4 C) to the colder surface (1–2 C).

Figure 11 illustrates the reduction in mean oxygen concentration immediately after the start of artificial circulation in 1963 and 1962. Patriarche (1961) suggested that reduced oxygen values were the result of upwelling bottom water that had a high B.O.D. Greenbank (1945) stated that hydrogen sulfide may remove dissolved oxygen from water by "chemical or biochemical oxidation or by the sweeping action of the gas in bubbling through the water." (Hydrogen sulfide is oxidized in the presence of oxygen (Hutchinson, 1957).) During artificial circulation, chemical oxidation of hydrogen sulfide is the more likely cause of oxygen reduction. Puke (1949) remarked on the danger to fish caused by reducing the oxygen concentration by the artificial introduction of hydrogen sulfide into surface waters already undersaturated with oxygen. In Corbett Lake, high B.O.D. and hydrogen sulfide were probably responsible for the initial reduction of dissolved oxygen. From November 10 to 18, 1962, the mean concentration of oxygen increased without the assistance of artificial circulation. The increase, however, occurred at a lesser rate than that of the

second or third period of artificial circulation. The greatest rate of increase occurred during the second period and was probably due to a drop in surface water temperature (Fig. 11, 1962). Artificial autumnal circulation thus provided a reservoir of dissolved oxygen in Corbett Lake that far exceeded that produced by natural circulation in the autumn of 1961 (Fig. 10). After artificial circulation, the dissolved oxygen concentration in Corbett Lake approached that naturally attained in Courtney Lake and was much greater than that naturally attained in Marquette Lake (Fig. 10).

Winter Limnology and Snow Cover

Light intensity and penetration markedly affect the production of photosynthetic oxygen in an ice-covered lake. Other workers have found that cloudy ice reduces light transmission (Croxton et al., 1937; Pounder, 1965; Greenbank, 1945; Woods, 1961; Wright, 1964). Fluctuations in dissolved oxygen concentration within one winter have been found to be associated with fluctuations of snow cover (Greenbank, 1945; Woods, 1961; Carufel, 1962). No such fluctuations in oxygen concentrations within one winter were observed during this study but differences in oxygen concentrations were evident between winters (Fig. 10, Marquette and Courtney lakes). The much-reduced snow and cloudy ice cover on Corbett Lake in 1962–63 resulted in an increase in light intensity and penetration compared with the winter of 1963–64 (Fig. 7). In Marquette and Courtney lakes the increase in light accounted for the formation of anomalous vertical temperature profiles (Fig. 8, March 7 and 8, 1963) and a discrete layer of supersaturated oxygen (Fig. 10, March 7 and 8, 1963). The layer of oxygen was probably produced by the dense layer of phytoplankton that was associated with the supersaturated oxygen layer. The phytoplankton layer was observed by scuba in Marquette Lake and was likely present in Courtney Lake as well.

The effect of varying amounts of snow-fall and over-winter survival or mortality of fishes has been well documented (Greenbank, 1945; Cooper and Washburn, 1946; Foster, 1960; Woods, 1961; Carufel, 1962). In all of these studies, complete, severe, or partial mortalities of fishes were associated with heavy snow covers and over-winter survivals with absent, periodic, or light snow covers. However, in this study "normal" snow-falls prevented any obvious addition of dissolved oxygen from a photosynthetic origin in the winters of 1961–62 and 1963–64. Thus, because of severe depletion of the limited autumnal oxygen reserve, all fishes in Corbett Lake in 1961–62 and in Marquette Lake in 1963–64 were subject to "winter-kill."

From these data it is possible to conclude that major factors causing periodic "winter-kills" of fishes in any one lake, are likely variations in the amount of snow and cloudy ice. In shallow, eutrophic lakes the winter oxygen demand may well be balanced or met by photosynthetically produced oxygen if there is sufficient light penetration through the snow and ice cover. However, many shallow eutrophic lakes are subject to successive "winter-kills"

because the B.O.D. consistently exceeds the autumnal reserve and/or winter production of oxygen.

Winter Oxygen Demand

The differences in oxygen consumption rate between the lakes and between winters are suggested by the durations, depths, and slopes of individual oxygen isopleths (Fig. 10). The slopes and depths of isopleths 0–6 mg/liter for Courtney Lake suggested a lower oxygen consumption rate than those of Corbett and Marquette lakes. In Courtney Lake, the slopes of these isopleths immediately following ice formation suggested an initially high consumption rate until about December 22 and then a lower, constant rate until ice break-up. Lower depletion rates in 1962–63 than in the other winters were probably the result of additions of photosynthetic oxygen.

The slopes of the oxygen isopleths for Corbett Lake were much steeper than those of Courtney Lake, suggesting a much greater rate of oxygen consumption. As in Courtney Lake, the greatest removal of oxygen in Corbett Lake was between freeze-over and about December 22. The slopes of isopleths 0–6 mg/liter for Corbett Lake in 1963–64 reflect an oxygen demand rate similar to that of 1961–62 whereas the slopes of isopleths for 1962–63 represent a much lower depletion rate which may reflect additions of photosynthetic oxygen.

The rates of oxygen depletion in Marquette Lake in 1963–64, and to a lesser degree in 1962–63, were similar to those in Corbett Lake in 1961–62. The severity of oxygen depletion in Marquette Lake was probably due in part to the poorer light-transmitting qualities of the water in Marquette Lake.

Whereas the natural autumnal circulation of Corbett Lake in 1961 resulted in low oxygen concentrations and consequent over-winter mortality of the fish population, its artificial circulation in the autumns of 1962 and 1963 resulted in a mean concentration of oxygen sufficiently high to prevent over-winter mortality during the winters of 1962–63 and 1963–64. In the winter of 1963–64, over-winter mortality of fishes did occur in Marquette Lake (incomplete natural autumnal circulation) whereas in Courtney Lake (complete natural autumnal circulation) over-winter mortality of fishes did not occur. The experimental circulation of Corbett Lake thus confirms that incomplete autumnal circulation and oxygenation can be responsible for over-winter fish mortalities.

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Hybridization and Isolating Mechanisms Between *Catostomus commersonii* and *C. macrocheilus* (Pisces: Catostomidae)[1,2]

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ABSTRACT

Catostomus commersonii and *C. macrocheilus* are sympatric in three major drainages of British Columbia. Morphological analysis employing a character index and discriminant function analysis of allopatric and sympatric populations revealed that hybrids were in 9 of the 11 lakes examined in which both species occurred. Presumed F_1 hybrids made up an average of 7% of the individuals of the combined populations where hybrids occurred. There was no evidence of backcrossing or swamping.

Segregation in spawning time and habitat was not apparent in four confined inlets which have migrations of both species. Some lakes supporting both species were found to have inlets in which only one species spawned. Temporal isolation was absent but habitat isolation was present in some areas of Cluculz River, an outlet with diversified habitat. Where segregation was apparent, *C. commersonii* spawned in shallow gravelly areas and *C. macrocheilus* in deep sandy areas. Artificial crosses of the species, two backcrosses involving hybrid females, and microscopic examination of hybrid testes failed to show any effective postmating isolating mechanism. An interaction of temporal, habitat, and postmating isolating mechanisms was not considered sufficient to prevent swamping. Ethological isolation is inferred to be of major importance in reducing initial hybridization. No explanation was found for the apparent failure of hybrids to successfully reproduce.

Hybridization could not be attributed to environmental factors commonly associated with this phenomenon. Males of one species entering the spawning act with males and a female of the other species is considered the main cause of hybridization. Mis-mated individuals, however, have much opportunity for conspecific matings at other times. There was no evidence of reinforcement of isolating mechanisms despite the probable occurrence of hybridization in some areas for at least several hundred years. Characters of each species were as distinctive in the areas of sympatry as in the areas of allopatry. Natural selection against individuals producing hybrids is apparently slow.

INTRODUCTION

Speciation may be considered complete when individuals of a population have acquired properties which enable them to maintain reproductive isolation from their parental species. Geographic isolation in sexually reproducing organisms is generally considered a prerequisite for the formation of these

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properties, which become tested when external barriers break down. The absence of adequate isolating mechanisms when populations come into secondary contact results in a fusion of the forms. The establishment of isolating mechanisms, which prevent interbreeding of sympatric populations, may thus be considered the most important aspect of speciation. The phenomenon of hybridization — defined here as the interbreeding of individuals belonging to two different species as a result of imperfect isolating mechanisms — presents, therefore, an intriguing problem in speciation.

Natural hybrids are known for many groups of animals (Mayr, 1963) and are relatively common among freshwater fishes in northern regions. The causes of hybridization are numerous and involve a breakdown of one or more isolating mechanisms. Hubbs (1955) stresses the role of environmental factors in inducing hybridization, and believes the relatively high rates of hybridization in northern freshwater fishes to be the result of the unstable environment. In certain cases hybridization has been attributed to the chance meeting of egg and sperm rather than to deliberate interspecific mating.

In several fishes some or most of the isolating mechanisms have been examined in detail and often with precise experimental work. Many studies discuss various facets of artificial hybridization, although most works on natural hybridization deal with cases where one or both of the parental species were introduced or where the environment had been altered by man. Most studies on isolating mechanisms in fish deal with species which never or rarely hybridize naturally. No previous study of fish examines most of the primary potential isolating mechanisms in which evidence is presented of two species hybridizing in many undisturbed localities throughout much of their area of sympatry.

In this study the hybridization and isolating mechanisms in *Catostomus commersonii*[4] (Lacépède), the white sucker, and *C. macrocheilus* Girard, the largescale sucker, were examined through field and experimental work. These two similar species of fish have come into sympatry in post-Pleistocene times and occur together in many British Columbia lakes. Hybridization between them has been reported by Carl et al. (1959) and Geen (MS, 1958) and is known from this study to be of wide occurrence. Their area of overlap is large, easily accessible to study, covers a wide range of environments, both disturbed and undisturbed, and has resulted from the natural expansion in range of both species.

There were three objectives to this study. The first was to establish a method of identifying hybrids and to determine the extent of hybridization.

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[4]According to the International Code of Zoological Nomenclature, 1964, 2nd ed., N. R. Stoll (Chairman), the original spelling of a name must be retained unless it is an inadvertent error [Article 32 a(ii)]. The original spelling of the white sucker, *commersonnii*, is apparently an incorrect spelling of Commerson, for whom the species was also inadvertently named after by Bernhard Germain Lacépède in 1803. The double "i" is not employed by some but appears to be required in the current rules. *Commersonii*, used by some workers, is adopted in this paper in preference to the commonly found versions *commersoni* and *commersonnii*.

The second objective was to study spawning populations and to make artificial crosses in order to evaluate the role of environmental factors in causing hybridization and to consider the effectiveness of the potential isolating mechanisms. With the above information, the third objective was to consider the biological significance of hybridization in these two species, and its bearing on their taxonomic status.

DESCRIPTION OF AREA STUDIED

GEOGRAPHY

The area studied lies within the zone of sympatry of *C. commersonii* and *C. macrocheilus* (Fig. 1), in the northern interior plateau of British Columbia. Most of the lakes and streams examined (Fig. 2) occur in the Endako (Skeena basin), Nechako (Fraser basin), and Crooked (Peace basin) river valleys between elevations of 640 and 762 m (2100 and 2500 ft) above sea level.

All creeks where spawning suckers were examined had a rapid flow over gravel. Cluculz River (Fig. 3–5), the outlet of Cluculz Lake, had an estimated flow of from 2000 to 3100 dm^3/sec (70–110 ft^3/sec) during late May, whereas the inlet creeks (e.g. Fig. 6 and 7) varied from an estimated 60 to 1700 dm^3/sec (2–60 ft^3/sec — see Table II). Freshets were common. There was little diversity in the small inlets except for short pools about 0.6–1.2 m (2–4 ft) deep, common along spawning areas in Decker, Stearns, Norman, Sucker, and Erickson creeks. Gravel from about 1.3 to 10 cm (0.5–4 inches) diam was common.

Farming practices have modified the banks adjacent to spawning areas in Palling, Decker, Richmond, and Stearns creeks. The substrate of these creeks has, however, been changed little. Burns Creek, which passes through the village of Burns Lake, was extremely polluted. The channels of Burns and Richmond creeks were disrupted in their lower courses. Cluculz River has been modified by a bulldozer in areas 6 and 7 (Fig. 3). Streams such as Corkscrew, Sucker, Norman, and Cluculz were poisoned in a sucker eradication program in the early 1950's. Except as noted above, the spawning areas for suckers have apparently been unmodified by man.

Information on the spawning migration in the diversified Cluculz River was collected in several locations. Depths and estimates of surface velocity in mid-river, obtained by timing a float over a fixed distance, were taken May 23–26, 1964. Temperature and depth records were measured in area 5. Velocity in the river varied from a low of 0.14 m/sec in area 1, the first area where suckers congregate for spawning, to a high of about 0.9 m/sec in areas 2 and 6. The diameter of stones was usually 3.2–130 mm. Water depth in mid-channel varied from 40 to 220 cm. Details of each area are given in Nelson (MS, 1965). The river for 1.6 km (1 mile) north of area 8 is similar to areas 1 to 8. Rapids exist farther downstream with falls, impassable to any upstream movement of suckers, near the junction with the Nechako River.

FIG. 1. Distribution of *Catostomus commersonii* and *C. macrocheilus* and accepted locality records in area of sympatry. Lake outlines are omitted. Doubtful literature records are indicated by question marks.

FISHES

Several species of fish occur in the streams during the spawning migration of *C. commersonii* and *C. macrocheilus*. *Catostomus catostomus*, the longnose sucker, precedes both species in spawning time and adults are usually absent when *C. commersonii* and *C. macrocheilus* are spawning. Considerable overlap in time does occur with *C. commersonii* in Norman Creek, but there are marked differences in areas of synchronous concentrations. Ripe *Mylocheilus caurinus*

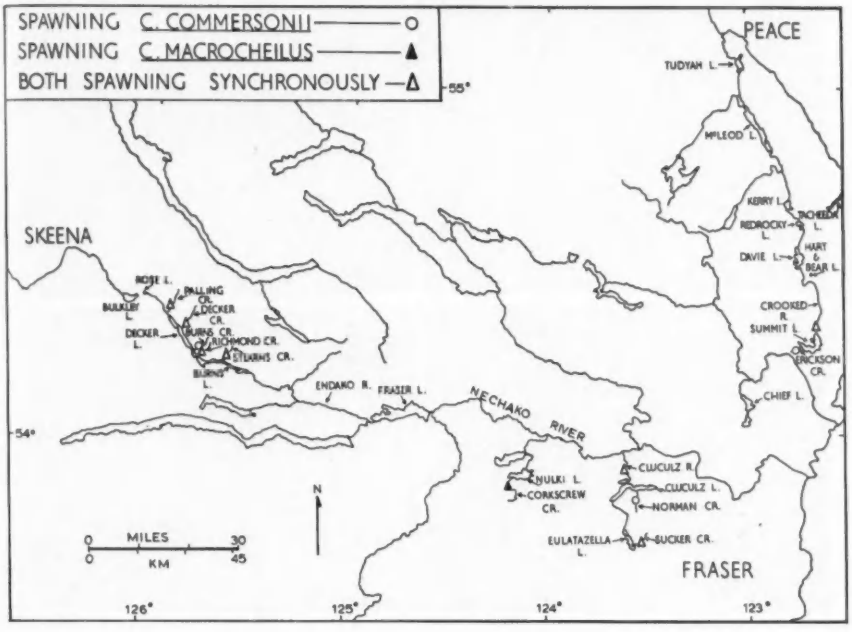

FIG. 2. Localities where spawning migrations of *C. commersonii* and *C. macrocheilus* were studied and areas examined for hybrids.

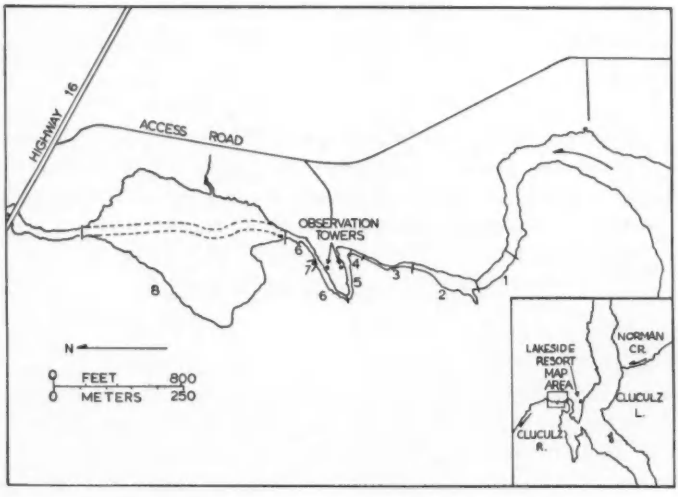

FIG. 3. Area selected to study temporal and habitat isolating mechanisms in Cluculz River.

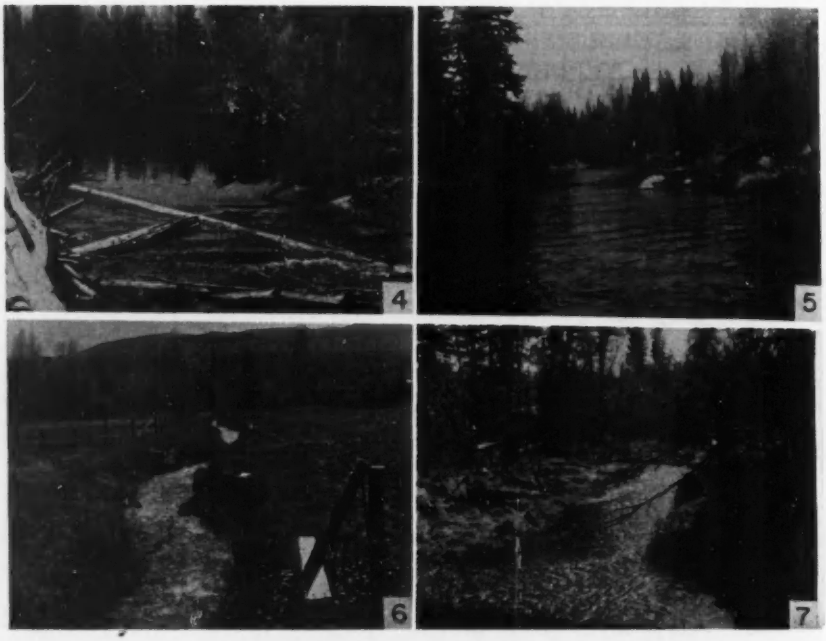

FIG. 4. Cluculz River with the riffles at end of area 2. May 25, 1964.

FIG. 5. Cluculz River in area 6. May 22, 1964.

FIG. 6. Richmond Creek at site where large numbers of ripe *C. commersonii* and *C. macrocheilus* were taken. June 2, 1964.

FIG. 7. Sucker Creek at site where large numbers of ripe *C. commersonii* and *C. macrocheilus* were taken. May 26, 1964.

is abundant during part of the sucker spawning migration in Decker, Burns, Richmond, Norman, Sucker, and Erickson creeks. *Richardsonius balteatus*, *Ptychocheilus oregonensis*, *Salmo gairdneri*, *Lota lota*, and *Cottus asper* are present in most of the creeks except Cluculz River.

MATERIALS AND METHODS

HYBRID IDENTIFICATION

MATERIAL EXAMINED

Material employed in the study included museum specimens, individuals collected during the study (Table I), and artificially reared individuals. All individuals used to establish the presence and to estimate the frequency of hybridization were seined and preserved without any conscious effort to favor particular types. Adults, except those gillnetted in Cluculz River, were obtained with rotenone (Chem Fish Special).

TABLE I. Localities from which suckers were examined for Fig. 9–16. Identifications are partly based on these figures. C: *Catostomus commersonii*; M: *C. macrocheilus*; H: Hybrids; BC: University of British Columbia; ROM: Royal Ontario Museum; NMC: National Museum of Canada; S or date: field number of uncatalogued material, some of which was discarded.

Locality	Lat N, long W	Museum No.	Total no. of specimens C, H, M	Fig.
Skeena R. drainage				
Owen L.	54°07′, 126°41′	S74	0,0,6	9
Collins L.	54°06′, 127°13′	BC61-479	0,0,7	9
Fraser R. drainage				
Burns L.	54°13′, 125°46′	BC64-416,427	17,6,8	12,16
Fraser L.	54°03′, 124°07′	NMC65-239,240 S1-64;BC64-409	0,4,83	16
Sucker Cr.	53°43′, 123°34′	BC64-260, 434	27,2,1	16
Norman Cr.	53°52′, 123°34′	June 9/63; June 4/64	91,0,1	16
Cluculz R.	53°54′, 123°35′	June 8/63; June 3/64; NMC65-241:BC64-408, 451	41,9,19	13,16
Chief L.	54°07′, 122°59′	BC64-404	28,4,2	16
Tyee L.	52°23′, 122°04′	S90	0,0,10	9
Duckworth L.	52°23′, 122°17′	S87	0,0,10	9
Anderson R.	50°42′, 121°57′	BC60-185,187	0,0,20	9,10
Schkam L.	49°25′, 121°27′	BC59-590;591;593	0,0,39	9,10,11
Lower Fraser R.	49°12′, 122°13′	BC59-601	0,0,19	9,10,11
Stave R.	49°12′, 122°23′	BC56-109,565	0,0,27	9,10
Nicola L.	50°12′, 120°29′	BC60-175;232	0,0,36	9,10
Shuswap L.	51°00′, 119°00′	BC54-349	0,0,3	10
Walker L.	50°33′, 120°09′	BC54-442	0,0,16	9,10
East Barriere L.	51°16′, 119°50′	BC58-300	0,0,3	9
Columbia R. drainage				
Missezula L.	49°47′, 120°30′	BC54-310	0,0,6	10
Allison L.	49°42′, 120°36′	BC56-580;59-311	0,0,21	9,10
Otter L.	49°34′, 120°46′	BC59-582	0,0,9	9,10
Wolfe L.	49°27′, 120°19′	BC56-89;57-297	0,0,42	9,10
Osoyoos L.	49°02′, 119°30′	BC53-187	0,0,19	9,10
Little Sand Cr.	49°20′, 115°18′	BC55-12	0,0,7	10
Kootenay L.	49°30′, 117°10′	BC57-57	0,0,10	9,10
Duck L.	49°14′, 116°38′	BC55-315	0,0,4	10
Bush L.	51°47′, 117°37′	BC56-475	0,0,5	9
Pend-d'Oreille R.	49°05′, 117°30′	BC56-523	0,0,18	9,10,11
Yakima R., Ellensburg, Wash.		BC58-241	0,0,9	9,10
Snake R., Pullman, Wash.		BC64-351	0,0,10	9,10,11
North Powder R., Haines, Ore.		BC56-66	0,0,6	10
Peace R. drainage				
Summit L.	54°17′, 122°38′	NMC65-253;254; BC64-400	56,2,19	14,16
Kerry L.	54°41′, 122°47′	NMC65-250	19,2,6	16
McLeod L.	54°56′, 122°59′	NMC65-249;BC64-397,398	28,5,50	15,16

(*Continued*)

TABLE I. Localities from which suckers were examined for Fig. 9–16. Identifications are partly based on these figures. C: *Catostomus commersonii*; M: *C. macrocheilus*; H: Hybrids; BC: University of British Columbia; ROM: Royal Ontario Museum; NMC: National Museum of Canada; S or date: field number of uncatalogued material, some of which was discarded. — (*Concluded*)

Locality	Lat N, long W	Museum No.	Total no. of specimens C, H, M	Fig.
Tudyah L.	55°04′, 123°02′	NMC65-248;BC64-396	56,2,3	16
Tacheeda L.	54°41′, 122°35′	July/62	12,0,0	9
Moberly L.	55°50′, 121°40′	NMC65-245	19,0,0	9,10
Charlie L.	56°15′, 120°58′	NMC65-244	11,0,0	9,10
Stoddart Cr.	56°14′, 120°56′	BC54-27	8,0,0	9
Tupper Cr.	55°38′, 120°04′	July/62	5,0,0	9
Swan L.	55°31′, 120°03′	BC61-312	10,0,0	9,11
Saskatchewan R. system				
White Mud Cr., Alta.		BC57-367	22,0,0	9,10,11
Meander R., Alta.		BC61-343	15,0,0	9,10
Shank Cr., Alta.		BC57-358	15,0,0	9,10
Unknown, Alta.		BC55-141	18,0,0	9,10
Eyebrow Cr., Sask.		BC61-96	6,0,0	9
Long R., Man.		BC58-93	15,0,0	9,10
Eastern Canada				
Unknown, Ont.		BC59-544	16,0,0	9,10
Lake of the Woods, Ont.		BC58 -380	15,0,0	9,10
Usher's Cr., Ont.		BC59-186	3,0,0	9
		ROM19691	6,0,0	9
Mykiss L., Ont.		BC58-374	3,0,0	9
Unknown, Ont.		BC:Uncat.	4,0,0	9
Boyer's Cr., Ont.		ROM19706	63,0,0	9,10
Near Owen Sound, Ont.		NMC60-499A	15,0,0	10
Nanticoke, Ont.		NMC60-527A	25,0,0	9,10,11
Cariboo R., N.S.		NMC59-292	32,0,0	9,10,11
Near Mud and Skiff lakes, N.B.		BC59-197	5,0,0	9,10

MORPHOLOGICAL COUNTS AND MEASUREMENTS

All body proportions were obtained from preserved material with dial calipers graduated and read to 0.1 mm. Magnification was used where desirable. Counts and measurements were taken on the left side of the specimen where possible.

The following characters were employed in the identification of individuals.

Dorsal to pelvic fin distance was measured from the origin of the dorsal fin (membrane over first rudimentary ray) to the insertion of the pelvic fin. Care was taken not to distort the body.

Caudal peduncle depth was measured at the narrowest place.

Dorsal ray count excluded the anterior two to four rudimentary rays. The last two ray elements, one branched and one unbranched, were counted as one. Alizarin Red S was used on small specimens.

It is felt that dorsal fin ray number was fixed in all specimens, both reared and natural, because there was no obvious correlation between fin ray number and fork length within the size range examined and because all specimens were greater than 20 mm fork length, the size at which MacPhee (1960) found dorsal ray number to be fixed in *C. macrocheilus*. A comparison of MacPhee's work with Stewart (1926) suggests that dorsal ray number in *C. commersonii* is fixed at a similar or earlier stage than in *C. macrocheilus*.

Gillraker count was the total number of anterior rakers counted under magnification on the dissected first left arch.

The number of gillrakers was counted over a wide length range of specimens and was found to be fixed at about 9 cm fork length. The asymptotic curve showed a rapid increase up to 7 cm. A correction factor was used so that a comparison between specimens of different lengths could be made. A count of two was added for fish $5\frac{1}{2}$–$6\frac{1}{2}$ cm and one was added to fish $6\frac{1}{2}$–8 cm fork length. Although this correction is crude, the error is not likely to affect the final results because species compared within a lake were of about the same size range and because the raker number was part of an index.

Lateral-line scale count was the total number of scales along the lateral line. It included scales on the caudal fin.

Diagonal scale count extended from the origin of the dorsal fin (anterior of the first rudimentary ray) downward and posterior to, but not including, the lateral line.

Peritoneal color was the degree of black pigmentation on the ventral abdominal cavity and was made using a series of standards for comparison. Four degrees were recognized. Scores were assigned with one given for absence of color and four to its full development. This character was useful only on specimens <15 cm.

Side color was the degree of black pigmentation in the form of three lateral blotches and was made using a series of standards for comparison. Four degrees were recognized. Scores were assigned with one given to full development of color and four to its absence. This character was useful only on specimens <15 cm.

Field Study of Species Segregation

Temporal and habitat segregation in *C. commersonii*, *C. macrocheilus*, and their hybrid were studied in the diversified Cluculz River and in several relatively uniform and confined inlets throughout most of the spawning period in 1963 and 1964.

In all streams the degree of female gonad development was noted (*ripe* eggs: near maximum size; cannot be extruded from cavity or can be extruded only with manual pressure; "hard" and yellowish — *fully ripe* eggs: spontaneously flow out from handling; jelly-like and orangish — *spawned*: few or no mature eggs left in body cavity).

Cluculz River

Gillnetting provided data on the relative abundance of the two species in eight areas. Observations from boat, shore, and towers provided comparative

data for each area. Observations, with the aid of binoculars and Polaroid glasses, were made of spawning acts.

Monofilament nylon gillnets were drifted for a distance of 0–6.1 m (20 ft) for 0.5–2 min on the river bottom. This method was effective in sampling segments of the spawning migration over short periods from the lake outlet to area 8 (Fig. 3). One or two nets, each 2.4 m (8 ft) deep and 15.2 m (50 ft) long of 6.35 cm (2.5 inches) stretched mesh were employed.

INLETS

Table II lists data on the inlets from which suckers were studied. Individuals were obtained with rotenone (Chem Fish Special) and collected in a seine stretched across the creek. A crude indication of the presence of any spatial isolation was obtained by subsampling in long stretches. Water temperature was measured between 1000 and 1600 hr.

Experimental Study of Species Segregation

HYBRID EMBRYO MORTALITY

(*a*) *Procedure.*— Embryo mortality tests were conducted at Lakeside Resort, Cluculz Lake (Fig. 3), from May 16 to June 9, 1963. Eggs and milt were obtained from suckers within 3 min of their capture in gillnets in Cluculz River. The procedure of fertilization was based on Davis (1953) and Forney (1957). Details are given in Nelson (MS, 1965). No fungus inhibitors or other chemicals were used. Lots were incubated in water from Cluculz Lake at temperatures similar to that where the donor specimens would have spawned.

There were 24 lots, each with 150 eggs, for the two homospecific and two heterospecific crosses. Each lot involved different parents and each of the four crosses was evenly distributed throughout the apparatus. The crosses were uneven in number because of an inability to collect desired parental types on any one day.

Eggs were placed in a single layer in 125-ml pyrex Erlenmeyer flasks and 10 × 10 × 15-cm baskets with an inner layer of nylon chiffon (40 meshes/cm) and an outer layer of nylon crinoline (10 meshes/cm) (Fig. 8). Water flow through each flask was 100–115 cc/min, which kept the lightest eggs in slow motion. The flasks received water siphoned from either of two 42-liter insulated aquaria. Six baskets containing eggs were placed in two rows in each of the above aquaria. Water was siphoned into these aquaria from two other tanks in which the cool water (7–12 C) pumped from Cluculz Lake was aerated and warmed by electric resistance heaters. Temperatures were recorded twice daily. The lowest and highest temperatures for the duration of the experiment were 8.5 and 13.5 C for the flasks and 9.5 and 15 C for the baskets.

Dead eggs were removed twice daily with a pipette and recorded. During the hatching period the number of hatched and unhatched eggs was recorded twice daily, without removing the eggs from their container. Mortality of newly hatched fish was recorded.

TABLE II. Inlets examined for spawning suckers from which data were utilized. Estimated volume of flow in dm^3/sec. P after date examined denotes *C. commersonii* or *C. macrocheilus* present; L denotes *C. catostomus* present; A denotes *Catostomus* sp. absent. Number in parentheses denotes number of times creek was examined.

Creek	Date examined	Temp (*C*)	Estimated vol. of flow	Area collected
Palling	May 29, 1963P	10	60	Highway 16
	June 2, 1964P	10	420	downstream for 200 m
Decker	May 29, 1963P	11.5	110	CN Railway
	June 2, 1963P	9.5	110	downstream to Decker L.
	June 2, 1964P	11	280	(500 m)
Burns	May 29, 1963P	13	60	Highway 16
	June 2, 1963P	10.5	60	downstream for 350 m
	June 1, 1964P	11.5	170	
Richmond	June 1, 1964P	15	140	Stretch for 100 m located on eastern limits of Burns L. townsite
Stearns	June 2, 1963P	8	170	From 50 to 250 m downstream from Highway 16
Corkscrew	May 13, 1962A	6		Stretch for 450–400 m
	May 22, 1962L	8.5	1680	from Nulki L.
	June 11, 1962P	10	1120	
	June 25, 1962P	15		
Sucker	May 12, 1962LP	10	170	Eulatazella L. upstream
	May 29, 1962P	13	220	for 300 m
	June 10, 1962P	12	140	
	July 15, 1962A	14	30	
	May 26, 1964LP	15		
	May 28, 1964LP	15	140	
Norman	May 10–30, 1962, (4)LP	10–15	220–140	Clucluz L. upstream for
	June 12, 1962A	14	110	1000 m
	July 30, 1962A	19	14	
	May 12–June 9, 1963, (7)LP	10–16	170–60	
	May 12–June 4, 1964, (6)LP	7–16		
Erickson	May 15, 1962P	9.5	220	Stretch for 1100–1000 m
	May 20, 1962P	13.5	220	from Summit L.
	May 26, 1962P	16.5		

Fully ripe eggs from the posterior ovary of 15 specimens of each species from Cluculz River and fertilized eggs of the four possible crosses at various ages were placed in a fixative of one part acetic acid and three parts ethanol (Clarke's fluid). The diameters of five eggs of each sample were measured with an ocular micrometer.

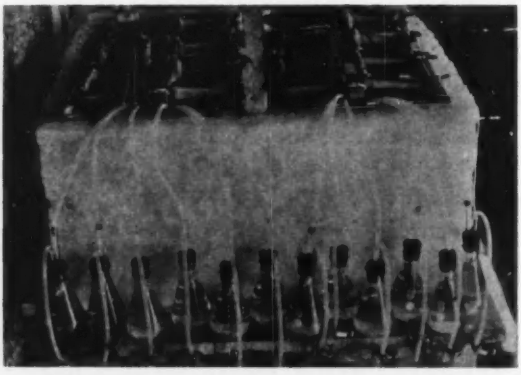

FIG. 8. Baskets and flasks of egg hatching apparatus for experimental crosses.

(*b*) *Treatment of data.*— In all calculations of mortality rate the arcsin per cent correction (Snedecor, 1956) was employed. The statistic t was used to test the significance of difference of means (Snedecor, 1956, p. 91). Hatching times were measured to note any differences between the species and their hybrids and any relation with mortality rate. Since the water temperature varied between days of incubation and between flasks and baskets and since the lots were started at different times, "days to hatching" must be converted to a measure which accounts for these variations. Thermal units (T.U.) measured in day centigrade degrees was chosen as the best approximation and is suitable for the present purpose. Hatching does not necessarily occur at a fixed developmental point (Embody, 1934) nor is the appearance of any anatomical feature of better value as a measure of developmental rate (Hayes et al., 1953). Hatching time was chosen because of its ease of measurement.

T.U. were calculated by measuring the area enclosed each day between 0 C and the temperature line, using an Ott compensating polar planimeter on a large-scale version of graphed temperature fluctuations. Biological zero was arbitrarily chosen at 0 C. The mean hatching time and variance were calculated (Nelson, MS, 1965).

HYBRID INVIABILITY

Certain lots (Table VIII) were reared beyond the experimental hatching. They were transferred to Vancouver from Cluculz Lake on June 12, 1963, and placed in holding tanks. Temperature was held between 17 and 18 C. The fish were fed protozoa (mostly *Paramecium* sp.), microworms, brine shrimp nauplii, frozen brine shrimp, and dried fish food.

HYBRID STERILITY

The offspring from two crosses involving probable hybrid females from Stearns Creek were reared in the Vancouver laboratory.

The testes of 21 presumed adult hybrids in spawning migrations from several localities were sectioned and stained with Ehrlich's haematoxylin. They were compared with similar preparations of *C. commersonii* and *C. macrocheilus*.

ZOOGEOGRAPHY OF *CATOSTOMUS COMMERSONII* AND *C. MACROCHEILUS*

Catostomus commersonii occurs in a wide variety of habitats across North America from Nova Scotia to British Columbia, and from Arkansas to the Northwest Territories. *Castostomus macrocheilus* is confined to western North America from the Sixes River, Oregon, to the Nass River, British Columbia, and to the headwaters of the Peace River system (Fig. 1).

The two species are sympatric in the upper Fraser, Peace, and Skeena drainages of British Columbia (Fig. 1). Both species also occur in allopatry within this general area of sympatry. In the southern area (Fraser River) *C. macrocheilus* is widespread whereas *C. commersonii* is surprisingly rare, known only in two lakes (Sixteenmile — 53°04′, 122°17′; and Green — 51°24′, 121°15′), from which *C. macrocheilus* is absent. In upper Peace drainages only *C. commersonii* has definitely been found in allopatry (Hart and Bear — shallow lakes with physical barriers in the outlets; and Tacheeda — a deep lake without physical barriers in the outlet). In the Nechako drainage both species occur in allopatry. Probably only *C. macrocheilus* occurs in allopatry in the Skeena system.

There is no apparent reason why some inlets of lakes in which both species are common support only *C. commersonii*, whereas both species occur in other inlets (Fig. 2). In Norman and Erickson creeks *C. macrocheilus* is rare, despite large numbers of *C. commersonii*. However, both species are common in Sucker, Stearns, Richmond, and Decker creeks, which are similar or smaller in size (Table II) than Norman and Erickson.

Gillnetting in Cluculz Lake (1962 and 1963) failed to show any difference in depth distribution or occupancy of different substrates between the two species. In all lakes seined no marked difference was found in relative numbers of suckers between times at the same locality. Despite the apparent lack of habitat difference in non-spawning suckers there was a marked difference between lakes in relative numbers of young seined. *Catostomus commersonii* was dominant in Rose and Tudyah lakes whereas *C. macrocheilus* was dominant in Fraser Lake. Generally, in the shallowest lakes *C. commersonii* occurred in greater numbers than *C. macrocheilus*.

An insight into the invasion routes followed by the two suckers and time of overlap has been gained from a study of their present distribution and by a knowledge of glacial geology (Holland, 1964; Drs W. H. Mathews and G. E. Rouse, personal communication). *Catostomus commersonii* probably had glacial refugia in both the Mississippi basin and Atlantic coast drainage, whereas *C. macrocheilus* persisted during the Pleistocene in the Columbia basin. At the end of the Wisconsin glaciation *C. commersonii* probably extended

its range westward into British Columbia through the Peace River. Access to Fraser drainage from the north possibly occurred through an outflow channel 10,000 or more years ago. Several other species of fish which had Mississippi refugia occur in the Peace River drainage, but those that are not in Crooked River drainage (headwaters of the Peace) are also not in Fraser drainage. Access to Skeena drainage from the Fraser would have been possible through swampy connections at Rose Lake and possibly at the old Stuart–Babine Lake portage route. The widespread occurrence of *C. commersonii* in the Nechako drainage suggests that ecological barriers, not recency of invasion, account for its absence from the Thompson and lower Fraser drainage. The nature of the barrier is unknown and it seems paradoxical that such a barrier exists when the wide tolerance of *C. commersonii* is considered. Several other species have a similar anomalous distribution in Fraser drainage, for which no explanation at present is without criticism.

Catostomus macrocheilus and several other species probably had access to Fraser drainage from their Columbia refugia through the low Shuswap–Okanagan divide or the outflow channel between the Nicola and Similkameen valleys. It cannot be established when *C. macrocheilus* occupied the upper Fraser. The most plausible hypothesis is that it occupied the Nechako basin, along with *C. commersonii*, at least several hundred, and perhaps several thousand years ago. It seems unlikely that *C. macrocheilus* could have been in the Nechako area some 10,000 or more years ago, when drainage was to the north, since glacial dams blocked the middle Fraser River prior to that date. Several species of fish which had a Columbia refugia, including *C. macrocheilus*, occur in McLeese Lake (52°24′, 122°20′) which has a high falls on the outlet upstream from the glacial block. The probable absence of *C. macrocheilus* in the Fraser during ice retreat suggests that access to McLeese Lake was probably gained by headwater capture from nearby Tyee Lake. Access to Peace drainage may have been through low areas, situated between Fraser and Parsnip drainage, east and south of Summit Lake (head of Crooked River). Failure to colonize the lower Peace River by *C. macrocheilus* and several other species has probably been due to ecological factors and is not evidence of a recent invasion. Access to Skeena drainage, as with *C. commersonii*, was probably through Rose Lake. That this or similar routes were followed by other species may be inferred from the many species (including all species that ascend inlets in the spring) shared between the Parsnip, Nechako, and Skeena rivers (see Lindsey, 1956).

Lack of adequate information on the relative times of drainage from post-glacial lakes and climatic conditions prevents sound conclusions regarding time of overlap. It would, however, be difficult to explain the distribution of the two species in the general area of sympatry if *C. macrocheilus* and *C. commersonii* did not have access to the Nechako basin at least several hundred years ago. Lakes probably persisted in the Peace area during the most recent glaciation, commencing about 25,000 years ago (W. H. Mathews, personal communication). Both species may have had access to the area, but

such a hypothesis requires more information. It is apparent from the distribution of these two suckers that the area of sympatry is a result of natural dispersion, and, particularly in the Nechako area, has existed at least for several hundred years.

IDENTIFICATION AND MORPHOLOGICAL ANALYSIS OF HYBRIDS

The identification of a natural hybrid is based on circumstantial evidence, usually morphological intermediacy between two species. Crosses made by plant and animal breeders in the last century showed that hybrids were generally intermediate to their parental species. Forms in nature intermediate between two species were subsequently interpreted as hybrids by some workers. Experimental studies showing that fish hybrids are usually intermediate (Hubbs, 1955) have given support to or have been the evidence for these interpretations.

The correct identification of hybrids is vital because of the varied importance ascribed to the role of hybridization in evolution. The presence of natural hybrids cannot be shown using conventional methods if sterile hybrids resemble one parental type through dominant inheritance. Also, interpretations other than that of hybrid origin exist for intermediate specimens. Firstly, the form may represent a species (*a*) ancestral to two divergent species, (*b*) established by hybrid origin but now self-maintained, or (*c*) intermediate in many respects between two species due to parallel evolution. Secondly, such a form could be of teratological origin and be "accidentally" intermediate in certain characters. Abnormalities are relatively common in Catostomidae. For example, a mature *C. macrocheilus* from Cluculz River had six dorsal fin rays and one anal fin ray (normal minima are 12 and 7 respectively); both fins appeared free of injury. Hubbs (1941) found several young *C. commersonii* to have a marked increase in the number of scales, overlapping the high number characteristic of *C. catostomus* with which it was sympatric. Lastly, an apparent hybrid could be a variant of a known species with characters which are at the extreme range and overlap those of another species. Such forms may exist in species not possessing known characters which separate the species by a distinct gap. White and Key (1957) found in a grasshopper that what had been interpreted as introgressive hybridization on morphological evidence was really an example of convergent evolution; all morphological intermediates could be placed in one of two species using cytological evidence with no indication of hybridization. Brown and Wilson (1956) point out that before a hybrid interpretation can be accepted the variation over the range of the presumed parental species must be known and the alternative explanation of displacement must be considered.

Rarity of distinctive specimens as evidence to substantiate a hybrid interpretation must be used with caution. Many species are rare in collections (but several previously described rare species or aberrant individuals are now considered hybrids). Conversely, hybrids need not be rare. Hubbs and Miller

(1943) found hybrids to constitute about 8% of the parental population in an entire basin.

Experimental crossing, although often providing conclusive evidence (e.g. Hubbs and Hubbs, 1932), cannot always be used to indicate what natural hybrids should look like, or when it can, does not necessarily verify the identity of a presumed natural hybrid. Crossing two individuals artificially need not yield offspring similar to those produced under natural conditions. Rough (MS, 1961) found counts in several meristic characters exceeding those of natural populations in crosses of *C. commersonii* at normal temperature (resembling *C. macrocheilus* in dorsal fin count). This difficulty can be circumvented by using controls (known pure crosses) in hybrid identification.

This study employed several criteria to reach conclusions on the extent of hybridization. The two species were examined throughout a large part of their area of allopatry and in allopatric populations within the area of sympatry, thereby establishing much of the variation of each character. Morphologically intermediate specimens were found only in those areas which contain both species and were considered to be hybrids. Reared offspring of experimental crosses provided information on the inheritance of certain characters in hybrids. The possibility of intermediate specimens representing a distinct species was eliminated by finding them sympatric only with both presumed parental species. It would be difficult to explain the zoogeography of a species confined to the glaciated interior of British Columbia. Evidence that potential isolating mechanisms would permit hybridization was obtained.

Analysis of Immature Specimens

Eight characters were selected from 62 characters which had been examined on 20 immature and 10 mature individuals seemingly to be of each species chosen from a sympatric population (Summit Lake). The selection of characters was based on their utility in separating the two species and the precision by which they could be measured. The characters were examined in many allopatric populations of both species and proved equally useful in separating the species.

The only character separating the species with a distinct gap was the ratio of the caudal peduncle depth to fork length. Dorsal to pelvic fin distance was chosen to form a ratio with caudal peduncle depth because it had a low correlation with caudal peduncle depth (0.52 and 0.21 in the 40 specimens each of *C. macrocheilus* and *C. commersonii*, respectively, of Fig. 11) and did in itself contribute to separating the species. A distinct gap exists between the two species in juvenile specimens (Fig. 9) and only specimens 5–18 cm fork length were used for the analysis of hybrids. Collections employed in Fig. 9 covered a wide range in fork length within each species and tended to overlap one another in the ratio with no suggestion of a cline. Allopatric populations within the area of sympatry (*C. macrocheilus* from Bulkley Lake (22 specimens) and Nulki Lake (48); *C. commersonii* from Tacheeda Lake (42) and Hart Lake (36)) fell within the range of their respective species in Fig. 9.

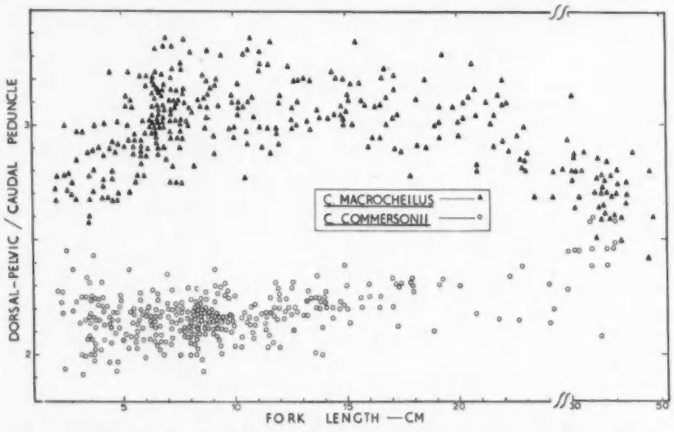

FIG. 9. Relation between ratio of dorsal-pelvic distance to caudal peduncle depth and fork length in allopatric *C. commersonii* (328 specimens, 22 populations) and *C. macrocheilus* (328 specimens, 20 populations).

Dorsal fin ray number was plotted against the above ratio (Fig. 10) in the graphical analysis to form the basis of hybrid identifications because (*a*) it was the only meristic character which could be examined in all fish (scales and gillrakers are often not countable in small specimens); (*b*) it is useful in separating the species and has a low correlation with the body ratio (0.33 and 0.38 in 40 specimens each of *C. macrocheilus* and *C. commersonii*, respectively, of Fig. 11); and (*c*) the available evidence from the artificial

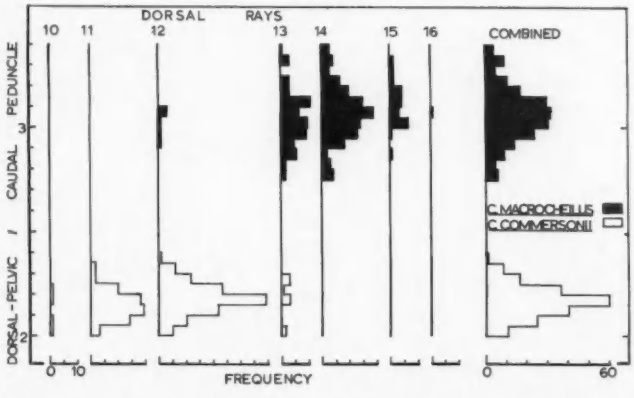

FIG. 10. Discrimination between allopatric *C. commersonii* (200 specimens, 14 populations) and *C. macrocheilus* (200 specimens, 19 populations) on basis of dorsal ray number and ratio of distance between dorsal and pelvic fins to caudal peduncle depth.

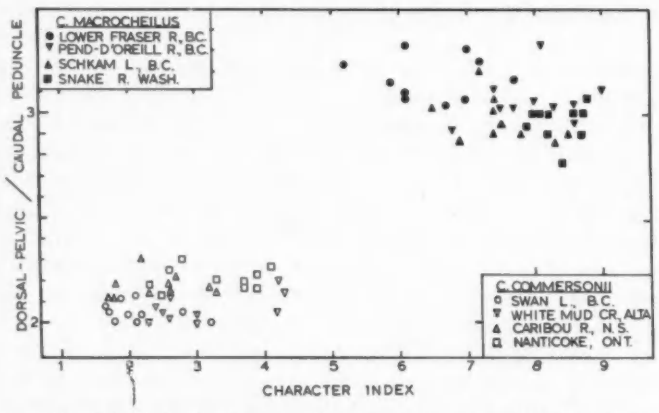

FIG. 11. Discrimination between allopatric *C. commersonii* (40 specimens, 4 populations) and *C. macrocheilus* (40 specimens, 4 populations). Character index calculated from gillrakers, lateral-line scales, diagonal scales, peritoneal color, and side color. Explanation of character index in text.

crosses showed that hybrids are intermediate in dorsal ray number (Table III). Specimens, 5.5–13.5 cm fork length, from populations both near to and far from the area of sympatry are in Fig. 10.

TABLE III. Inheritance of dorsal ray number. Species, number of rays, and sex of parent given under cross. C: *C. commersonii*; M: *C. macrocheilus*; H: probable hybrid.

Cross	10	11	12	13	14	15	Mean
C12♂ ×M15♀	0	0	2	9	2	2	13.2
C11♂ ×M14♀	0	1	12	24	11	2	13.0
C11♀ ×M15♂	0	1	2	4	2	0	12.7
C10♀ ×M14♂	1	0	0	0	0	0	10
C11♂ ×C12♀	0	4	5	0	0	0	11.5
C11♂ ×H12♀	0	3	24	1	0	0	11.9

Preliminary analysis revealed that some specimens in sympatric lakes had a body ratio which fell within the gap of allopatric specimens. Since there was no basis for knowing whether hybrids overlap the parental species in this body ratio another independent analysis was made. A character complex involving five characters was found suitable. Of the five characters, lateral-line scales could be counted in 19 artificial hybrids. Their number was intermediate between the species. The highest correlation coefficient within 40 specimens of each species comparing pairs of the seven characters was 0.55, obtained in *C. macrocheilus* between diagonal and lateral-line scales. Only

6 of 42 correlations had a r value > 0.36 and were significantly different from zero at the 0.02 level. The index was based on individuals from five sympatric lakes. Individuals were chosen which had a body ratio within and varying throughout the full range of the species. The inclusion of hybrids would give conservative conclusions, that is, identify fewer hybrids than exist.

The combined range of measurements for each character for equal numbers (total of from 80 to 140 for each character) of both species was divided into 20 parts with equal numbers of individuals in each 20th. A score of 0–10 was given to each count according to which 20th it occupied. The low and high counts correspond to *C. commersonii* and *C. macrocheilus*, respectively. The index for each specimen was the mean of the five characters (three for Burns Lake).

In allopatric populations (Fig. 11) the index produced complete separation of the two species, whereas each component character overlapped between species. All immature specimens in the area of sympatry were identified using a combination of Fig. 10 and 11 (e.g. Fig. 12–15). Some specimens had a body

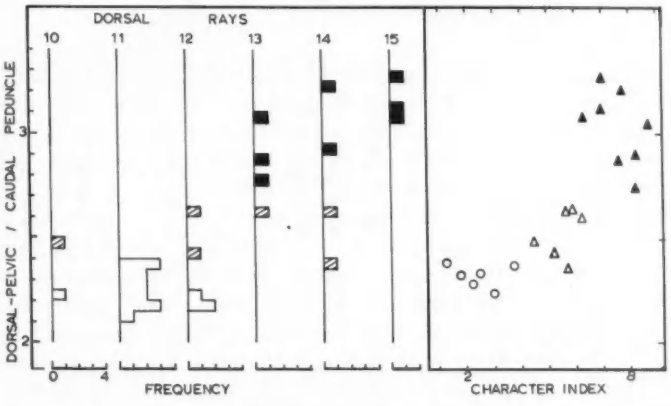

FIG. 12. Character analysis used to identify immature *C. commersonii* (open histograms, open circles for selected individuals), *C. macrocheilus* (solid histograms, solid triangles for selected individuals), and hybrids (hatched histograms, open triangles) in Burns Lake. Character index calculated from gillrakers, peritoneal color, and side color.

ratio higher or lower than the species gap, but had a character index tending in the opposite direction. Such specimens, never found in allopatry, were considered hybrids. Specimens in the character index include, where possible, all specimens with a body ratio lying in the gap between the parental species as established in Fig. 9. Specimens above and below the area of intermediacy were examined until only the parental species were left. A sample of each species was also included in the figures. Results from all areas of sympatry are summarized in Fig. 16.

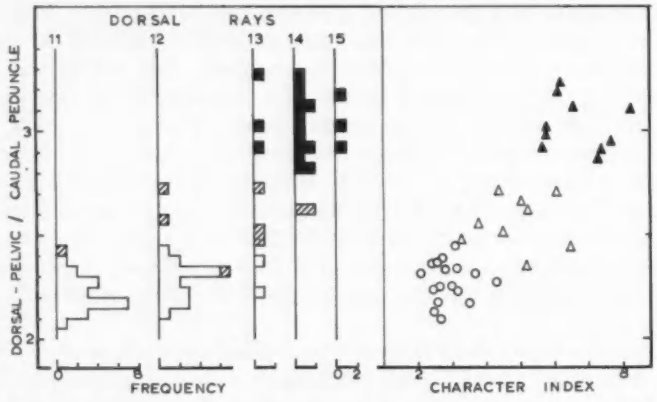

FIG. 13. Character analysis used to identify immature *C. commersonii*, *C. macrocheilus*, and hybrids (explanation of symbols in Fig. 12) in Cluculz River. Character index calculated from gillrakers, lateral-line scales, diagonal scales, peritoneal color, and side color.

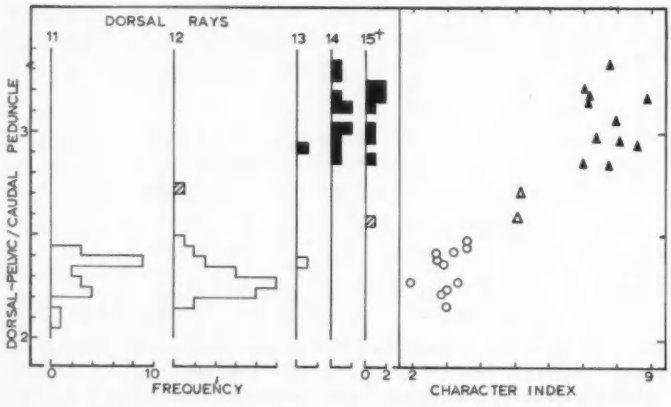

FIG. 14. Character analysis used to identify immature *C. commersonii*, *C. macrocheilus*, and hybrids (explanation of symbols in Fig. 12) in Summit Lake. Character index same as in Fig. 13.

The percentage of immature sucker hybrids varied from 17% in a sample of 31 from Burns Lake (Fig. 12) and 13% in a sample of 69 from Cluculz River (Fig. 13) to 2.5% in a sample of 77 from Summit Lake (Fig. 12). Of the total sample of 499 suckers from areas where hybrids were found, 7.2% were hybrids. These hybrids constitute 13.2% and 18.8% of the specimens, respectively, of *C. commersonii* and *C. macrocheilus*. Both species were caught without hybrids in only three areas: Rose Lake with 117 *C. commersonii* and 1 *C.*

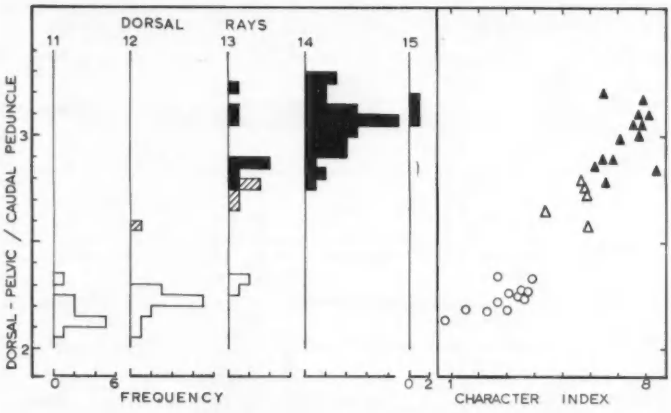

FIG. 15. Character analysis used to identify immature *C. commersonii*, *C. macrocheilus*, and hybrids (explanation of symbols in Fig. 12) in McLeod Lake. Character index same as in Fig. 13.

macrocheilus; Norman Creek with 81 *C. commersonii* and 1 *C. macrocheilus*; and Davie Lake with 9 *C. commersonii* and 13 *C. macrocheilus*. With the inclusion of these latter populations hybrids constitute 5% of all immature specimens. Hybrids were taken only in one lake without both species in the sample; several hundred *C. macrocheilus* but no *C. commersonii* were seined from Fraser Lake. The latter species exists in tributaries and probably occurs in Fraser Lake in low numbers. Bias may arise in per cents if the species and hybrids segregate. No obvious difference in species ratio was noted within lakes and within Cluculz River where several seine hauls were made at different times and areas. However, in Sucker Creek, poisoning revealed 27 immature *C. commersonii* and 1 *C. macrocheilus*, whereas amongst adults the latter species was more abundant (1962 year class — Fig. 22). Earlier poisoning here and in Norman Creek suggests that at least immature *C. commersonii* may ascend inlets.

Each character used in the index was intermediate in the hybrids (Fig. 17 and 18) with considerable spread into the parental species. The indices employed for identification (column 1, Table IV) were recalculated using data of Fig. 17 and 18 (column 2, Table IV). Substantial differences were noted only for lateral-line scales. Using the recalculated index altered the index scale and occasionally the relative position of individuals, but did not affect conclusions regarding identification of individuals.

To further evaluate the mathematically crude graphical index, a discriminant function analysis (Williams, 1959) involving five different functions, each with differing numbers of characters, was made on an IBM 7040 computer. The following data were calculated: (i) correlation coefficients of characters within and between each species from a total of eight allopatric populations;

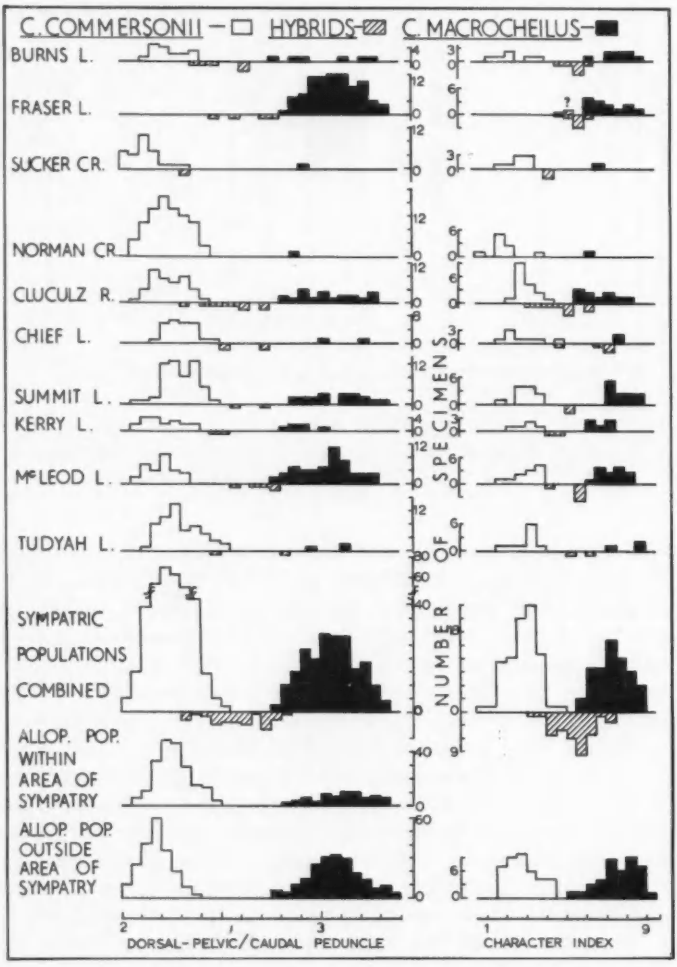

FIG. 16. Summary of dorsal-pelvic distance to caudal peduncle ratio and character indices used to identify immature hybrids. Specimens used in the body ratio represent random collections.

(ii) correlation coefficients of the five discriminant functions between and within each species in allopatry; (iii) evaluation of usefulness of each character in discriminant functions; (iv) theoretical and empirically derived calculations on utility of separation of each character; (v) discriminant functions for each specimen in eight allopatric populations and Cluculz, Summit, and McLeod

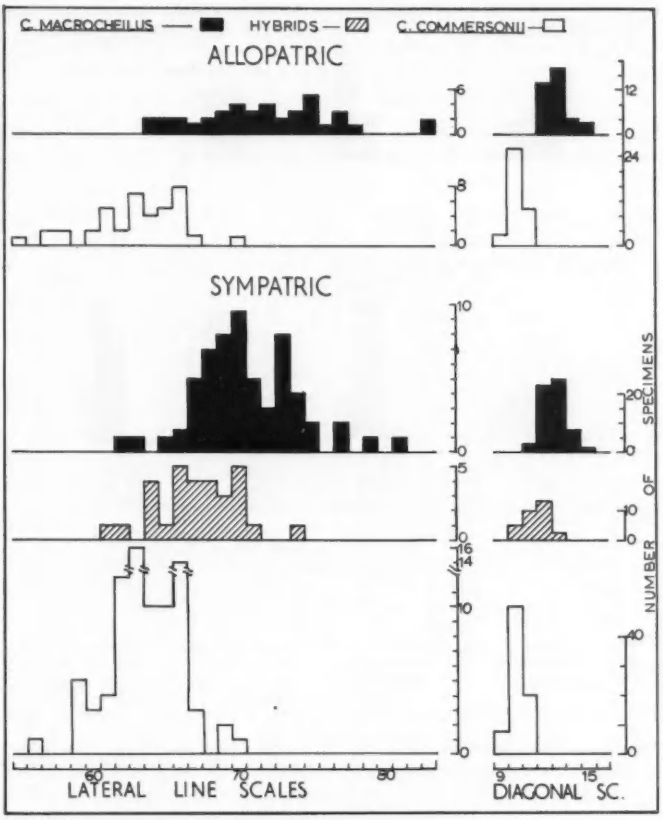

FIG. 17. Distribution of lateral-line scales and diagonal scales in *C. macrochelius*, hybrids, and *C. commersonii*. Allopatric data (*top*) comprise the four allopatric populations of both species of Fig. 11. Sympatric data (*bottom*) comprise specimens whose identity was determined by body ratio and character index. Only lake systems where hybrids were found are included.

lakes; (vi) average discriminate scores for each allopatric population; and (vii) discrimination between species for each analysis.

There was complete agreement, except for one specimen, in classification of specimens between the graphical character index and treatment of the same characters in the discriminant function analysis. The discriminant analysis provided better separation of the species in allopatry than the index in which characters were given equal weight. However, as in the graphical analysis, the subjective interpretation on some borderline specimens remained. Different interpretations would have been made in a few cases if fewer or more

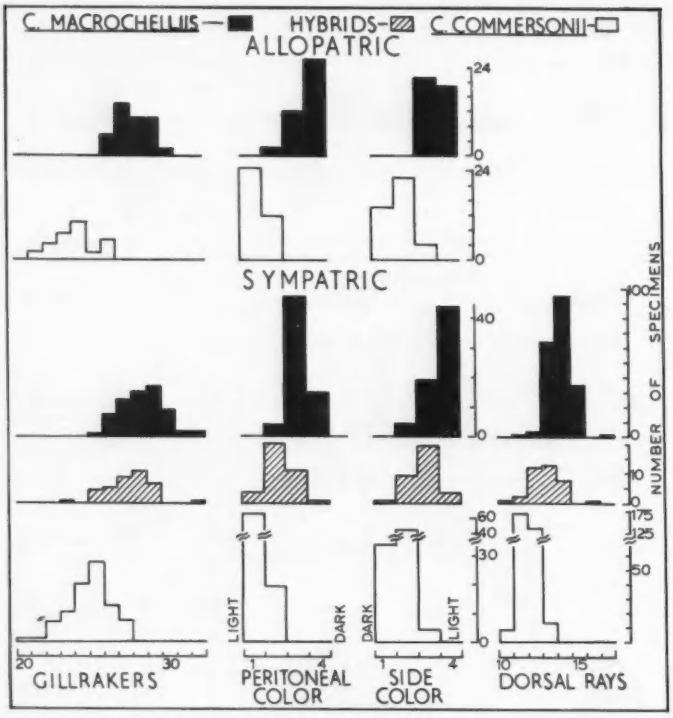

FIG. 18. Distribution of gillrakers, peritoneal color, side color, and dorsal rays in *C. macrocheilus*, hybrids, and *C. commersonii*. See Fig. 17 for further explanation.

characters had been used, but the relative numbers of hybrids to parental types would not have been changed. The easily computed graphical analysis was therefore considered sufficiently accurate in identifying individual hybrids. The morphological distinctiveness and intermediacy of certain specimens, and their occurrence only in areas occupied by the presumed parental species, left no reasonable doubt as to their hybrid origin.

ANALYSIS OF ADULT SPECIMENS

The identification of adult hybrids, in contrast with immature, was somewhat uncertain. Adults could not be separated into two distinct groups plus some independently tested intermediates, using characters employed on immature specimens, since body proportion, side color, and peritoneal color were of little or no value in separating adults.

When five or more characters were employed there was little difference in the mean discriminant function scores within a species between the allopatric

TABLE IV. Index values assigned to five characters. Side and peritoneal color indices combined. Column 1 denotes indices employed in analysis (Fig. 11–15 and 19). Column 2 gives recalculations of indices from results of column 1 (Fig. 17 and 18). Dorsal rays are included.

Diagonal scales			Lateral-line scales					
No.	1	2	No.	1	2	No.	1	2
9	1.2	0.4	60	0.2	0.5	71	6.9	8.2
10	3.3	3.7	61	0.7	0.7	72	7.2	8.5
11	5.4	5.3	62	1.3	1.6	73	7.8	9.2
12	7.0	7.2	63	1.8	2.6	74	8.3	9.5
13	8.7	9.3	64	2.4	3.2	75	8.7	9.7
14	9.7	9.9	65	3.4	4.0	76	9.2	9.7
15	10	10	66	3.9	4.9	77	9.5	9.8
			67	4.4	5.4	78	9.7	9.8
			68	4.9	6.2	79	9.9	9.9
			69	5.8	6.9	80	9.9	9.9
			70	6.7	7.8	81	10	10

Gillrakers			Color			Dorsal rays	
No.	1	2	No.	1	2	No.	2
22	0.7	0.5	2	0	1.4	10	0.1
23	1.4	1.2	3	2.5	4.1	11	2.4
24	2.6	2.3	4	3.8	4.9	12	4.7
25	3.6	3.9	5	5	5.3	13	6.7
26	4.3	5.1	6	6.2	6.5	14	9.0
27	5.2	6.6	7	7.5	9.3	15	9.9
28	6.5	7.8	8	10	10	16	10
29	7.7	9.2					
30	9.0	9.6					
31	9.7	9.7					

populations of Fig. 11, but when only diagonal scales, lateral-line scales, and dorsal rays were considered, there was much difference. For example, *C. macrocheilus* in Fraser River (BC 59-601) were intermediate between *C. macrocheilus* of Snake River (BC 64-351) and typical *C. commersonii*. No marked difference, however, occurred within a species when using populations from the area of sympatry. This allowed a common score to be used for all populations.

Scores based on three meristic characters applied to allopatric specimens, known sympatric immature specimens, and unknown adult specimens, suggested the presence of many adult hybrids amongst this latter group (Fig. 19). No attempt was made to include or exclude particular specimens since

scores based on extremes of each species may indicate "hybrids" as an artifact of the method.

Adults were accepted as presumed hybrids in later sections only if they had a meristic combination which was more probably that of a hybrid than of the pure species (based on Fig. 19 plus gillraker counts for preserved spec-

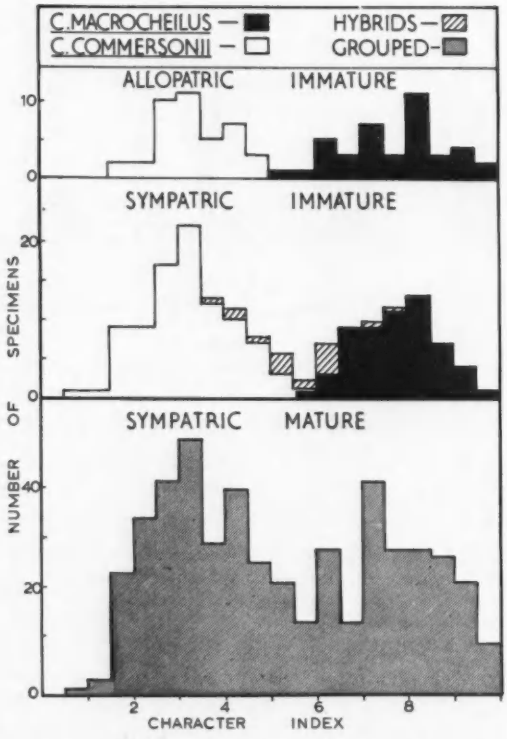

FIG. 19. Comparison of mature sympatric suckers with immature allopatric (of Fig. 11) and sympatric suckers (of Fig. 16) to show the existence of mature hybrids. Number of hybrids made proportionate to pure types. Sympatric specimens (*bottom*) are from spawning migrations in Palling, Decker, Burns, Richmond, Stearns, Cluculz, and Norman streams. Character index based on dorsal rays, diagonal scales, and lateral-line scales.

imens). The per cent of presumed hybrids of the sampled population was 15 in Decker Creek; 6–9 in Palling, Richmond, Stearns, and Sucker creeks; and about 2.5 in Burns, Norman, and Erickson creeks and Cluculz River.

ISOLATING MECHANISMS

Temporal Isolation

Many congeneric species and even sympatric "races" are isolated only or in part by differences in time of breeding. The purpose of this section is to determine the extent of any differences in time of the spawning migration of the two species in sympatry.

Adult suckers generally occupy the streams only during their spawning migration. Temperature appears to be the most important factor in initiating the spawning migration. This is suggested by the correlation of temperature with commencement of the sucker migration both between years within a particular stream and between streams within a particular year. Water temperatures in mid-May were lower in 1964 than 1963 in the inlet (Norman Creek) and the outlet of Cluculz Lake (Fig. 21). The spawning migration was delayed in both streams in 1964 (Fig. 20 for Cluculz River). The spawning migration of *C. commersonii* in Cluculz River was earlier, and temperatures were lower in 1956 and 1957 (Geen, MS, 1958) than during the present study. Migrations in Corkscrew Creek were several weeks later than in nearby warmer inlets (1962 observations – Table II).

CLUCULZ RIVER

Catostomus commersonii and *C. macrocheilus* begin their spawning migration at about the same time (Fig. 20). In 1964 the river had been extensively examined for several days before the first suckers were observed below area 1. In 1963 gillnetting and shore observations indicated that *C. macrocheilus* was present for a longer time than *C. commersonii*. Up to and shortly after May 28 the number of suckers was decreasing; the number present on June 8 and 10 suggested the presence of a second migration of *C. macrocheilus* (Fig. 20) which had not yet spawned (Fig. 21).

The most meaningful measure of temporal isolation is not the timing of spawning migrations, but the overlap in the time of fully ripe individuals in the spawning area. Females were used to indicate the amount of temporal overlap between the two species because they were present for a shorter period of time than males. In 1963 and 1964 females of both species became fully ripe at about the same time (Fig. 21). Fully ripe females of both species were present synchronously for at least 12 days, virtually the entire period in which there were fully ripe *C. commersonii*.

Males of both species had freely running milt and spawning coloration before the females were fully ripe. In 1963 and 1964 there was evidence that fully ripe *C. commersonii* females outnumbered similar *C. macrocheilus* females (Fig. 21) at the beginning of their spawning migration, whereas an opposite relation held at the end. Fully ripe males of both species were common throughout the period.

Although suckers may spawn mainly at night much spawning activity was observed in daylight in the present study (Cluculz River for both species

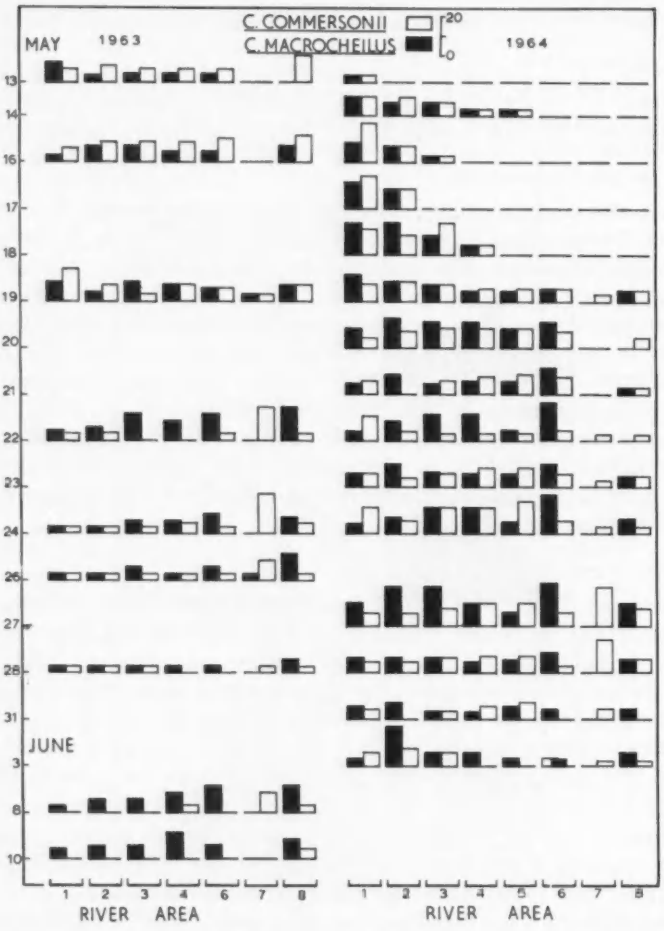

FIG. 20. Distribution of adult suckers in Cluculz River. Areas correspond to Fig. 3. Ratio of two species within each area calculated from gillnet catches. Differences between days in area 6 based on catch per unit effort (scale corrected to catch in two nets in about 1.5 min). Visual observations and gillnet catch used to determine variation between areas within days. Catch in all cases equals or exceeds that given in figure.

under both cloudy and sunny conditions, McLeese and Tyee lakes for *C. macrocheilus*, Bear Lake for *C. commersonii*).

INLETS

Evidence from inlets further suggests that there is little temporal isolation between the two species. The duration of the spawning migration was estab-

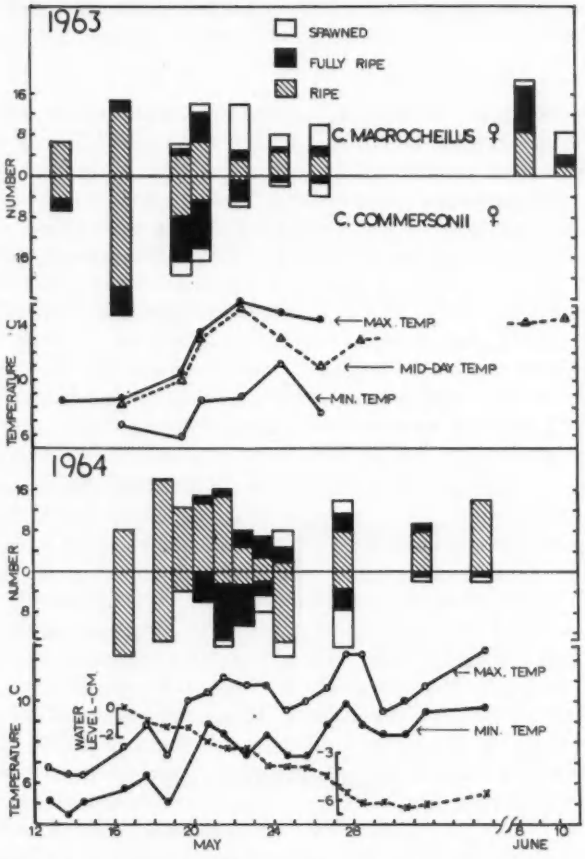

FIG. 21. Degree of female maturity and water temperature in Cluculz River for 1963 and 1964. Mid-day temperatures recorded between 1300 and 1500 hr. See Materials and Methods for explanation of degrees of maturity.

lished only for Norman Creek, in which *C. macrocheilus* adults were rare. Fully ripe females of both species were present at the same time in all creeks (Fig. 22) in which both species were found (adult or immature). It is almost certain that the creeks in which only *C. commersonii* was found do not have runs of *C. macrocheilus* at another time even though these creeks (Burns, Norman, and Erickson) drain into lakes where *C. macrocheilus* is abundant.

HABITAT ISOLATION

Selection of different areas for spawning often acts as a mechanism for reproductive segregation (Hubbs, 1961). The purpose of this section is to

examine areas of occurrence of suckers in Cluculz River and certain inlets to determine if there is any spatial isolation between the two species.

CLUCULZ RIVER

Marked differences in area of occurrence were apparent from gillnet catches of the spawning population (Fig. 20). Data from May 13 to 28, 1963, and May 13 to June 3, 1964, were analyzed (Model I, analysis of variance). During this time both species were about equally common in the river. Significant differences occurred in areas occupied both in 1963 ($F_{6,36} = 4.15$, $p < 0.005$) and 1964 ($F_{7,98} = 6.12$, $p < 0.005$). In 1963 the greatest difference was attributed to the predominance of *C. commersonii* in area 7 and of *C. macrocheilus* in areas 3 and 6. In 1964 the greatest difference was attributed to the predominance of *C. commersonii* in area 7 and of *C. macrocheilus* in areas 2 and 6. In 1963 the marked difference in area 3 had no isolative value because equal numbers of fully ripe females of both species were captured on the same day. In 1964 *C. commersonii* was rarer than *C. macrocheilus* in area 2, but had a greater number of fully ripe females (16 compared with 5, respectively).

The greatest consistent segregation in fully ripe individuals was in areas 6 and 7. Only two fully ripe *C. commersonii* compared with 14 fully ripe *C. macrocheilus* females were collected in the main channel of area 6 in both years. The *C. commersonii* females were caught before the *C. macrocheilus*. From May 25 to 28, 1964, at times between 0830 and 1130 hr, 332 matings of primarily *C. macrocheilus* (pairing of males with a female in which one or more males pass through a series of tremors) were observed in area 6. Most matings were at depths from 60 to 82 cm. Males preferred the swifter offshore water while females occurred near shore. Prior to mating the females moved toward midstream. At least five matings were observed which involved the smaller *C. commersonii* males from adjacent area 7. Observation and gillnetting in 1963 and 1964 showed that only *C. commersonii* spawned in area 7 (May 21 to 24, 1963, and May 27 to 29, 1964). Spawning was over gravel at depths usually less than 30 cm. No large suckers resembling *C. macrocheilus* were observed or caught in area 7 while *C. commersonii* spawned there. Substantial spawning apparently occurred in these two adjacent areas with very few interspecific matings.

In some areas other than 6 and 7 an interaction of temporal and habitat isolation produced effective segregation. Fully ripe *C. macrocheilus* predominated in area 8 and fully ripe *C. commersonii* predominated in area 5.

Figure 20 and additional data suggest that where segregation occurred *C. commersonii* spawned over coarse gravel in relatively shallow water whereas *C. macrocheilus* spawned over sand or fine gravel in relatively deep water. When *C. macrocheilus* occupied the river in the absence of ripe *C. commersonii* (early June, 1963), it showed the same preference to areas as when *C. commersonii* was present.

Presumed hybrids occurred throughout the spawning migration and in all areas except 7. Fully ripe hybrid individuals of both sexes were taken in

close proximity with each other and with fully ripe individuals of the pure species.

INLETS

There was no evidence of species segregation in inlets where *C. commersonii* and *C. macrocheilus* were common (Fig. 22). Subsampling over apparently uniform substrate (the uniformity of most inlets relative to sucker size did not allow a breakdown into discrete areas) showed no obvious differences.

Several creeks had only one common species but drained into lakes containing both species. Sampling in Palling Creek produced 1 *C. commersonii*

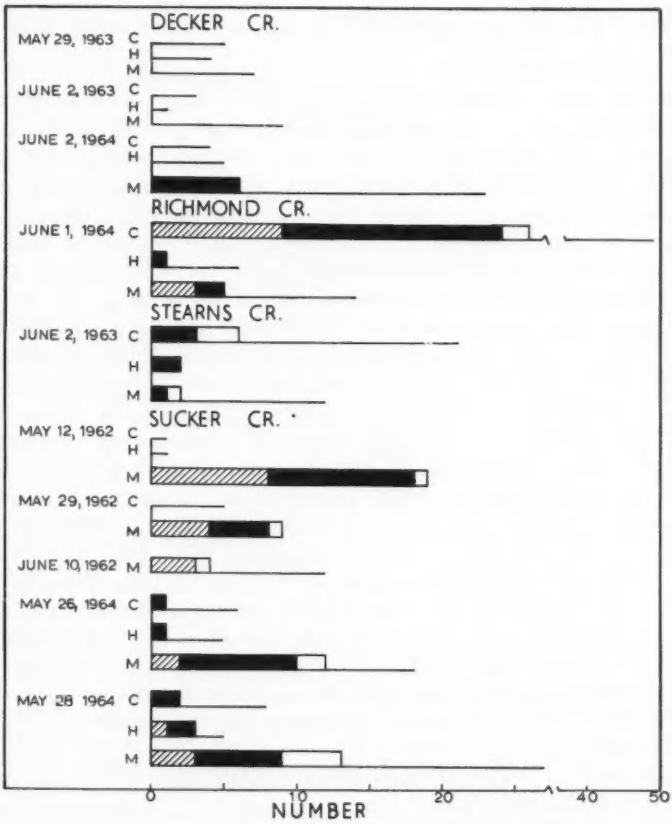

FIG. 22. Relative abundance of *C. commersonii* (C), presumed hybrids (H), and *C. macrocheilus* (M) in inlet spawning migrations where both species were common. Number of females indicated by bar, number of males indicated by line starting from 0. Stearns Creek had one hybrid male. Proportionate number of females in various degree of maturity indicated as in Fig. 21.

and 28 *C. macrocheilus*. Sampling in Burns Creek produced 64 small *C. commersonii* but no *C. macrocheilus*. Some 307 *C. commersonii* and 5 *C. macrocheilus* were collected throughout the spawning run for 3 years in Norman Creek. *Catostomus macrocheilus* adults occurred off the mouth of this inlet (1962 data) but ascended it rarely. Geen (MS, 1958) also noted *C. commersonii* in inlets of Cluculz Lake but did not find *C. macrocheilus*. Erickson Creek had 91 *C. commersonii* and 1 *C. macrocheilus*. Burns Creek is much shallower than the other inlets. Because *C. macrocheilus* possibly cannot become stunted to the degree of *C. commersonii* (Fig. 23), mechanical selectivity acting on size may prohibit the entry of *C. macrocheilus* from this shallow stream. Creek size (Table II) does seem related to the size of migrants (Fig. 23). No explanation can be given for the disproportionate numbers in inlets other than Burns.

GENERAL CONSIDERATIONS

Although only *C. commersonii* spawned in a shallow gravel area of Cluculz River and fully ripe *C. macrocheilus* outnumbered *C. commersonii* in deep channels with sand or fine gravel, both species spawn in diversified habitats throughout their range. They spawn in inlets and outlets with differing physical conditions and appear to overlap the spawning habitats of each other. *Catostomus commersonii* apparently seldom or never spawns in deep water with a sand bottom, although in eastern North America, Raney (1943) has observed it to spawn in a relatively deep quiet pool with gravel.

Spawning requirements of *C. commersonii* are generally considered to be relatively shallow water running over a gravel bottom (Reighard, 1915, 1920; Geen, MS, 1958), but the species is not so restricted. Lake spawning was observed in allopatric *C. commersonii* in Bear Lake May 26, 1962. There is also evidence for lake spawning in *C. commersonii* in eastern North America (Geen, MS, 1958). *Catostomus macrocheilus* spawns in deep sandy areas of Cluculz River and in shallow coarse gravel inlets. Allopatric *C. macrocheilus* were observed to spawn over gravel in McLeese Lake from 0700 to 1030 hr on June 14 to 18, 1962. Water depth was 0.3–1.2 m (1–4 ft). Fully ripe and spawned males and females were gillnetted from the site. Lake spawning also occurred in Tyee Lake on June 18, 1962, over gravel areas. In addition, allopatric populations have been found spawning in outlets (Geen, MS, 1958) and on lake margins and in inlets (Weisel, 1957). Keating (personal communication) found egg deposition by *C. macrocheilus* at the lower end of pools in an Idaho stream at 1.2–1.8 m (4–6 ft) depths, in primarily sand with 5–10 cm (2–4 inch) rubble.

In sympatry, reproductive segregation was not apparent in the confined inlets. In the diversified Cluculz River, in general, *C. commersonii* preferred shallow gravel areas whereas *C. macrocheilus* preferred deep sandy areas.

Ethological Isolation

Of the several mechanisms usually operative which prevent interbreeding of animal species, ethological isolation is often the singly most important

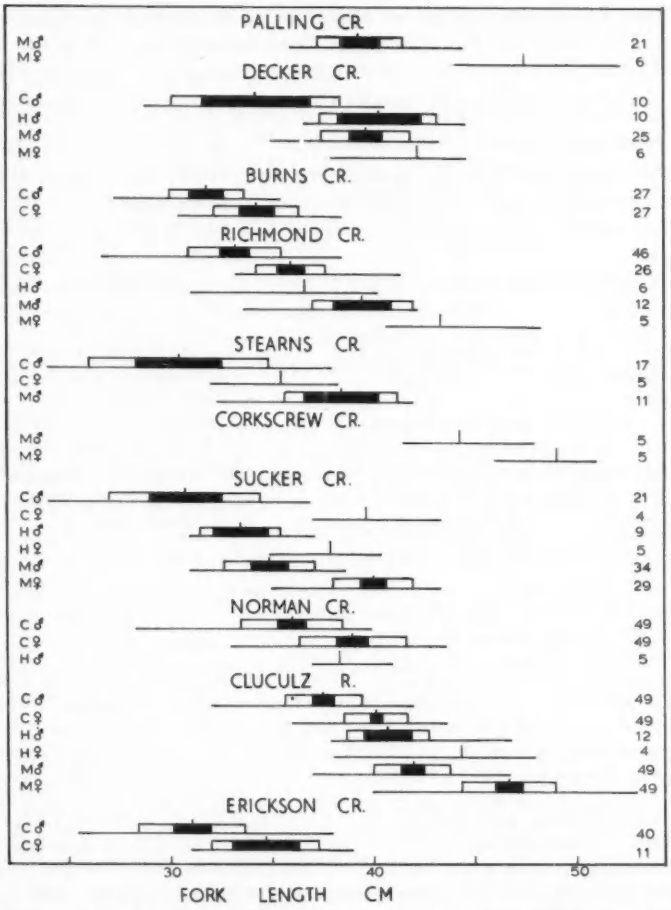

FIG. 23. Fork length of *C. commersonii* (C), presumed hybrids (H), and *C. macrocheilus* (M) in spawning migrations. Numbers within streams do not necessarily reflect relative abundance. Sub-samples are random in fork length. Black bar denotes four standard errors of the mean. Black bar plus white bar on either side of the mean outlines one standard deviation. Horizontal line represents range. Sample size shown at right.

(Mayr, 1963). Useful results on the relative strength of conspecific and interspecific matings were not obtained in mate choice tests conducted in fenced areas of Cluculz River, adjacent to areas of natural spawning.

Many studies invoke ethological isolation to be of major importance in isolation but relatively few studies actually demonstrate the mechanism. Picciolo (1964) found visual stimuli the most important of several possible

mechanisms for discrimination in four species of anabantids. Hanson (MS, 1965) provides evidence that *Oncorhynchus nerka* tend to select mates of about their own size. The possibility of color, morphological, and size differences being a basis for favoring conspecific matings was therefore explored.

COLOR DIFFERENCES

Color differences were studied on freshly caught fish held under water and by observing undisturbed known fish. Individuals were used from Cluculz River and nearby inlets. A summary of differences is given below.

Catostomus macrocheilus	*C. commersonii*
Females	
1. Lateral and dorsal body surfaces greenish yellow to golden when mature.	1. Lateral and dorsal body surfaces uniform steel gray or grayish hue when fully ripe
2. Faint to distinct black band, when fully ripe, extending ventrally from the lateral line for 4–5½ scale rows, extending anteriorly under the eye and around the snout, and posteriorly to caudal peduncle.	2, 3, 4. No distinct black, yellowish, or olive green bands present (yellowish white band reported in their eastern range by Reighard, 1920).
3. Above black band distinct in spawning individuals.	
4. Band 3–5 scale rows wide, dorsal to the lateral line, iridescent olive green to ochre.	
5. Narrow yellowish stripe below the black band and above the ventral whitish surface.	5. No yellowish stripe above the ventral whitish surface.
6. Anterior margin of pectoral and pelvic fins usually dark.	6. Anterior margin of pectoral and pelvic fins usually white.
Males	
1. Resemble *C. macrocheilus* females except the black and olive green bands develop sooner and last longer; black band a deeper black (jet black) than in females.	1. Resemble *C. commersonii* females up to time when milt can be expressed; brownish gray tinge sometimes present. Fully ripe males resemble *C. macrocheilus* males. No bright reddish stripe (in place of the dark stripe) was observed in spawning individuals although it has been noted for the species in their eastern range (Reighard, 1920).
2. Anterior margin of pectoral and pelvic fins usually dark, but occasionally white.	2. Anterior margin of pectoral and pelvic fins usually white.

MORPHOLOGICAL DIFFERENCES

Interspecific differences exist in dorsal fin length, head shape, body shape, and number of scales. Morphological sexual differences exist but are similar

in both species (e.g. mature males have tubercles and distinctively shaped caudal, anal, pelvic, and dorsal fins).

SIZE DIFFERENCES

Within each species, females are usually longer than males, so that in conspecific matings males would be expected to mate with females of a larger size. *Catostomus macrocheilus* is longer than *C. commersonii* in sympatry, but there is much overlap (Fig. 23). Interspecific matings could readily occur in one reciprocal cross with the smaller male to larger female relation still maintained.

Reighard (1920) describes the position of males and females in the pairing act in *C. commersonii*. It is not known how important the relative positions are to successful spawning; however, the body contacts as described by Reighard (1920) could probably not occur if males were larger than the females or much smaller.

Hybrid Embryo Mortality

Congeneric species of fish can usually be crossed (Hubbs, 1961) although marked differences in mortality between reciprocals are known (Hubbs and Drewry, 1959). The hypothesis that *C. commersonii* and *C. macrocheilus* are not sufficiently dissimilar in their genetic constitution to restrict the production of first generation hybrids was tested.

There was assumed to be no differential mortality rate as a result of starting day of the crosses. Comparable lots did not suggest otherwise. Egg mortality generally occurred throughout the rearing period with no obvious difference in the time of death between the four crosses. Pre-hatching mortality rate was not significantly different between flasks and baskets in the 16 comparable lots ($t_{14} = 0.23$, $p > 0.8$). Flasks and baskets were thus combined in calculations.

No significant difference occurred in pre-hatching mortality among the four crosses (Tables V, VI, and VII). The hybrid cross with *C. commersonii* females, however, had the highest mean mortality both within flasks and baskets. The highest t value of the four crosses was obtained comparing the two reciprocal crosses and was not significant ($t_{10} = 1.89$, $p \geq 0.08$). In addition to reflecting differences in the viability of zygotes, the variation in mortality between lots may reflect differences in stripping technique and condition of the parents.

Hatching time was not significantly different between flasks and baskets in the 16 comparable lots ($t_{14} = 1.31$, $p > 0.2$). Flasks and baskets were thus combined in calculations. Analysis of variance of the mean hatching time showed little difference ($F_{3,23} = 3.07$, $p > 0.05$). The hatching time of the hybrid cross with *C. commersonii* eggs was significantly shorter than either its reciprocal ($t_{10} = 2.6$, $p < 0.05$) or the *C. commersonii* control ($t_{13} = 2.7$, $p < 0.02$). Differences among all other combinations were not significant. A tendency for hybrid reciprocals to approach their maternal parents was noted for darters by Hubbs and Strawn (1957a, p. 50) and Hubbs (1961a).

TABLE V. Hatching time and mortality of the crosses in flasks. Hatching time in day degrees C. Each lot contains 150 eggs. C: *C. commersonii*, M: *C. macrocheilus*.

	Cross											
	CC						C♀M♂			C♂M♀		MM
No. hatched:	113	116	93	123	110	136	104	103	106	121	122	110
Mean hatching time:	195	182	188	169	174	178	170	159	154	199	178	207
Hatching time SD:	16.8	20.2	16.1	16.6	13.5	21.4	10.2	12.5	11.0	8.7	13.5	17.3
Per cent 3-day mortality:	39	31	21	24	57	0	20	28	28	0	11	0
Combined per cent mortality:	54	46	51	38	68	9	44	51	49	18	28	26

No causal relation can be attached to the hybrid cross with *C. commersonii* eggs having both the shortest hatching time and a tendency to have the highest mortality rate. There was no significant correlation between the number of eggs hatching and the mean hatching time, either within crosses or with crosses combined. Hatching frequency data are given in Nelson (MS, 1965).

The diameter of unfertilized non-swollen *C. commersonii* eggs ($\bar{X}$ = 2.1 mm in Clarke's fluid) was significantly smaller (t_{13} = 10.4, $p < 0.001$) than those of *C. macrocheilus* ($\bar{X}$ = 2.5 mm). This same relation held after fertilization. Eggs from hybrids between the two species were intermediate in size. The hybrid cross involving *C. commersonii* females had smaller eggs than its reciprocal in the four embryonic stages examined. The tendency for

TABLE VI. Hatching time and mortality of the crosses in baskets. See Table V for explanation

	Cross											
	CC			C♀M♂			C♂M♀				MM	
No. hatched:	104	132	121	47	118	115	119	126	110	114	112	122
Mean hatching time:	168	174	160	152	172	162	174	161	177	181	167	174
Hatching time SD:	11.5	15.8	6.1	8.1	14.3	13.0	7.1	9.1	21.0	14.8	18.0	16.5
Per cent 3-day mortality:	25	37	0	10	69	13	4	3	30	41	15	23
Combined per cent mortality:	48	44	19	72	76	34	24	18	49	55	36	38

TABLE VII. Summary of statistics for comparing mortality rates of the crosses. Number of lots shown in parentheses after cross. See Table V for legend.

	Cross			
	CC (9)	C♀M♂ (6)	C♂M♀ (6)	MM (3)
Pre-hatching mortality				
Mean (arcsin $\sqrt{\%}$)	27.8	35.5	27.1	29.0
Sum of squares	323	541	36.7	17.3
Corrected mean (%)	21.8	33.7	20.8	23.5
Three day post-hatching mortality				
Mean (arcsin $\sqrt{\%}$)	27.2	31.0	18.7	17.3
Sum of squares	2248	912	1101	469
Corrected mean (%)	20.9	26.6	10.3	8.9
Combined mortality				
Mean (arcsin $\sqrt{\%}$)	39.9	47.8	33.8	35.3
Sum of squares	1099	469	487	29
Corrected mean (%)	41.2	54.9	31.0	33.4

the smaller egged reciprocal to have the highest mortality rate is not necessarily a causal relation since opposite findings are known from other fish (Hubbs and Drewry, 1959).

Hubbs (1960) provided evidence that sperm of two species of *Etheostoma* are more capable of fertilizing conspecific eggs than interspecific eggs after a time lapse, thereby selecting against accidental hybridization. No such tests were done in this study. Certainly gametic incompatability was not found to occur without a time lapse. Samples of eggs were taken from the pan used for fertilization and placed in flasks and baskets without conscious effort to favor particular types. Of the 150 eggs finally put in each container, only a maximum of 12 per lot and a total of 161 eggs for all lots were rejected as showing signs of being abnormal. There was no obvious association with this low number and the type of cross or the pre-hatching mortality of each lot. Selection against hybridization could thus not be shown to be present on the basis of selective fertilization or embryo mortality.

HYBRID INVIABILITY AND STERILITY

Many fish species produce fertile and viable hybrids without losing their genetic identity (Hubbs, 1961). The possibility that hybrids, once produced, may be at a selective disadvantage was explored.

HYBRID INVIABILITY

(*a*) *Post-hatching mortality.*— A measure of post-hatching mortality was obtained by noting the number of hatched young to die 3 days after mean

hatching time in each lot. Mortality was not significantly different between flasks and baskets in the comparable lots ($t_{14} = 0.3$, $p > 0.7$) nor was the mortality from fertilization to 3 days after mean hatching ($t_{14} = 0.2$, $p > 0.08$). Flasks and baskets were thus combined in calculations.

Post-hatching mortality per cents are based on unequal sample sizes caused by pre-hatching mortality. The error involved in using these per cents, however, does not affect the conclusion that the tendency for the hybrid cross with *C. commersonii* females to have the highest mortality rate was not significant. The highest t value was obtained in comparing the reciprocals ($t_{10} = 1.5$, $p > 0.1$).

The hybrid cross with *C. commersonii* females had a significantly higher mortality than its reciprocal ($t_{10} = 2.48$, $p < 0.05$) from fertilization to 3 days after hatching. Neither hybrid cross was significantly different from the controls (conspecific crosses). No significant difference occurred between hybrids and the control crosses ($t_{22} = 1.3$, $p > 0.2$). The tendency for hybrids with *C. commersonii* females to have a higher mortality (Table VII) than the controls was balanced by the opposite tendency of its reciprocal.

There was no suggestion of selection against the F_1 hybrids in the lots transferred to the Vancouver laboratory (Table VIII). However, 5 out of 19 hybrids

TABLE VIII. Survival of offspring of artificial crosses in laboratory. Species and sex of parent given under cross. Eggs in the last two crosses hatched after transfer. C: *C. commersonii*; M: *C. macrocheilus*; H: probable hybrid.

Cross	No. hatched	No. alive at transfer to laboratory	Survival; days after transfer				
			15	68	118	201	281
C♂ × M♀	126	110	51	18	9	6	3
C♂ × M♀	122	108	102	52	41	20	6
C♀ × M♂	103	55	45	10	6	0	0
C♀ × M♂	115	88	45	1	1	1	1
C♂ × C♀	116	80	55	9	2	1	1
C♂ × H♀	119	132	103	38	28	15	2
C♂ × H♀	90	108	38	9	2	0	0

longer than 36.5 mm from one cross with a *C. macrocheilus* female had a deformed mouth. The opening was reduced and terminal with the upper jaw compressed. The mouth had moved ventrally in other hybrids at this size. Deformed hybrids died but the cross nevertheless had the highest overall survival rate. The deformity was not observed in several hundred similar-sized suckers from Cluculz River.

Laboratory hybrids as well as the pure cross and naturally hatched offspring had similar habits. They remained on the bottom (naturally hatched

offspring were under gravel) for the first few weeks until the elongate yolk sac was absorbed. They then became primarily pelagic; several months later, after their mouths had moved ventrally, they usually fed on the bottom.

(*b*) *Ratio of young to adult natural hybrids.*— Any progressive change in proportion of hybrids within a year-class would provide a measure of hybrid inviability. Problems of aging and sampling did not allow changes between years in hybrid proportions in a year-class to be studied. The same information could be obtained by noting differences in per cent hybrids between year-classes in any year, if per cent hybridization was the same each year for a particular area. There is, however, no reason to believe the incidence of hybridization is constant. A crude measure of natural inviability from immature to the mature stage was obtained by grouping samples comprising several year-classes, including both immature and adults, from several areas.

The inference from Fig. 19 that hybrids between the immature and adult stage are at a selective advantage cannot be accepted, because the several assumptions needed to reach the conclusion are probably not true. There is no evidence, however, that there is selection *against* individual hybrids. The per cent of presumed adult hybrids through individual analysis comprised 4% of the total population where hybrids were found.

HYBRID STERILITY

(*a*) *Crosses.*— Two crosses which very probably involved hybrid females (fork length (cm): 35, 32; dorsal rays: 12, 12; lateral-line scales: 74, 71; diagonal scales: 11, 11; gillrakers: 26, 27, respectively) and *C. commersonii* males (fork length: 36, 28; dorsal rays: 11, 12, lateral-line scales: 64, 66; diagonal scales: 9, 10; gillrakers: 23, 26, respectively) showed no obvious difference between mortality of their offspring and of other crosses (Table VIII). Individuals of the most successful cross were reared up to 39 mm fork length with one individual reared to 56 mm. No obvious morphological deformities were noted in the young.

(*b*) *Gonads.*— Both sexes were found in presumed adult hybrids (53 males and 21 females). This unequal sex ratio is commonly found in collections from spawning populations of the parental species and does not suggest a disturbed sex ratio. All stages of maturity (ripe, fully ripe, and spawned) occurred in presumed hybrids. Milt and eggs could be expressed from hybrids as in the pure species.

The gonads of hybrids had the same external appearance and appeared as large as the parental types. Much variation, however, exists. Recently spawned individuals had a few large ova (about 2.4 mm and orangish) whereas the remainder of the ovary had small ova (0.1–1.2 mm and whitish), which again is similar to the parental species. Sections of testes from 21 presumed hybrids showed no noticeable difference from the parental species. Both unspawned males with tubules full of gametes and spawned individuals with few or no gametes and collapsed tubules occurred.

DISCUSSION

Extent of Hybridization

Hybridization between *C. commersonii* and *C. macrocheilus* occurs in at least nine lakes, representing a wide diversity of environments throughout much of the area of sympatry and all but two of the lakes where a sample was obtained. Different proportions of immature hybrids in various lakes, ranging from 2.5 to 17% of all suckers sampled, may not reflect differences in rate of hybridization but be attributable to different selective pressures against young hybrids, or to sampling error. The only consistent trend noted was a tendency for per cents to be higher in lakes in the Fraser River than in the Peace River basin.

Specimens difficult to classify may be products of backcrossing, but analysis of the dispersion of hybrid characters (Fig. 16, 17, and 18) suggests that backcrosses are absent or very limited. If backcrossing did occur more frequently than initial hybridization, then borderline specimens should be more numerous than they are. It is doubtful, however, if individual backcrosses could be differentiated (even by methods of Anderson, 1953, and others) from variants of the species, unless such backcrosses were common and the various recombinants not selected against. The presence of backcrosses would not be indicated even if a high variability existed in the hybrids (Atz, 1962; Hubbs and Strawn, 1957b), especially if any were produced by delayed fertilization from stray sperm which causes increased variability (Hubbs, 1957).

The extent of hybridization in these two suckers does not suggest they are not full species. Various studies (Gilbert, 1961, and Hubbs, 1961 for fish; Cory and Manion, 1955 for amphibians; Sibley and West, 1958 for birds; Mayr, 1963 for other groups) describe two forms failing to hybridize in some areas but having mass hybridization in other areas, with various transitional cases often present, suggesting the existence of pairs which bridge the gap between good species and conspecific populations. Many hybridize only under environmental disturbances. The presence of hybrids does not necessarily indicate that isolating mechanisms are not keeping the parental species segregated. Hybrid swarms associated with environmental disturbances no more demonstrate conspecificity than do the many artificial crosses which have shown that related fish can readily produce viable and fertile hybrids (Hubbs, 1961; Hubbs and Drewry, 1959). If all evidence suggests that two forms are remaining distinct in at least one locality, regardless of whether hybrids or hybrid swarms occur elsewhere, the forms, when distinguishable, should be recognized as distinct species. If there is maintenance or divergence of differences in the area of hybridization, without immigration, the process of speciation may be considered complete. This is in agreement with the biological species concept given by Mayr (1963).

Catostomus commersonii and *C. macrocheilus* probably represent an irreversible discontinuity. There is evidence from many varied environments that their status as distinct species (from one another), which has never been questioned, is valid.

Causes of Hybridization

Environmental Factors

The environmental factors that Hubbs et al. (1943) and Hubbs (1955, 1961) note as having weakened isolating mechanisms and allowed hybridization do not appear to have caused hybridization between *C. commersonii* and *C. macrocheilus*.

(*a*) *Disproportionate numbers.*— Stebbins (1959) notes that unequal numbers of individuals between plant species have long been known to be conducive to hybridization. In animals, hybridization has often been attributed to the failure of an individual to find a conspecific mate (e.g. Hubbs, 1951; Hubbs et al., 1943; Hubbs and Miller, 1953; Hubbs and Laritz, 1961; Mecham, 1960; Sibley, 1961). Brower's (1959) data on butterflies can be similarly interpreted.

Unlike finds of other studies, the present results showed that inlets (Norman and Erickson) and lakes (Rose, Fraser, and Tudyah) which had marked differences in relative numbers of the two species had few hybrids. Inlets with relatively equal numbers of the two parental species (Decker, Richmond, and Stearns) had the highest per cent of hybrids.

If only conspecific matings occur when a choice of mate exists, but hybridization occurs when there is no choice, the expected result would be an inverted V-like curve if per cent hybrids is plotted against species ratio. Factors such as number of encounters required with the opposite species before interspecific matings occurred would influence the shape of the curve. However, such a simple relation does not appear to exist in the present data.

Another explanation of the apparent discrepancy between this study and that of others is suggested by the data from inlets. An increase in hybridization with a decrease in disproportion of the two species might be explained if habitat segregation were important and ethological segregation weak. In small inlets, a similarity in numbers of each species would result in maximum hybridization, whereas in a diversified environment, such as Cluculz River, habitat segregation would keep hybridization low. However, if ethological segregation was weak the rate of hybridization would probably be higher than was actually observed.

Hybridization of these suckers thus does not appear to be explained by a large disproportion in numbers of spawners. In Cluculz River the disproportion in fully ripe individuals of both sexes between each species at the beginning and at the end of the spawning migration, however, may have been conducive to hybridization.

(*b*) *Restricted spawning area.*— Restricted spawning area which forces species to mate in close proximity is often associated with hybridization (e.g. Bailey and Lagler, 1938; Trautman, 1948). There is no evidence of spawning area being restricted or having been decreased in the past where *C. commersonii* and *C. macrocheilus* are sympatric. Crowding of individuals suggests the possibility of accidental fertilization, a widely invoked cause of hybridization

(Hubbs, 1955; Hubbs and Miller, 1953; Miller, 1964). Although laboratory experiments have demonstrated that sperm longevity will permit accidental fertilization (Hubbs, 1957, 1960, 1961b), field experiments testing the hypothesis are lacking.

Hubbs (1957, 1960, 1961b) and Hubbs and Drewry (1958) have found gametic incompatability after a time lapse which would reduce the chances of accidental interspecific fertilization. Since delayed fertilization may result in lower hatching rates, short temporal function of sperm may be a significant isolating mechanism in restricted spawning areas.

(*c*) *Disturbances or intergradation of the environment.*— Many cases of hybridization, both in plants and animals, have been attributed to disturbances of the environment (Anderson, 1953; Hubbs, 1955, 1961; Mecham, 1960; Sibley, 1961). Much of the area of hybridization in suckers has not been disturbed by man and inlets where disturbances have occurred show no obvious higher per cent of hybrids than undisturbed inlets. One of the most pronounced areas of habitat segregation occurred in the disturbed section of Cluculz River, areas 6 and 7. Man's activities have provided a shallow gravel bank where *C. commersonii* spawned separately from *C. macrocheilus*.

(*d*) *Species introduction.*— Hybridization often appears attributable to the introduction of one or both parental species into a new environment (Hubbs and Hubbs, 1947; Hubbs et al., 1943). Species which hybridize when introduced into the range of another often occur together naturally elsewhere without hybridizing (Hubbs, 1961). Overlap in *Catostomus commersonii* and *C. macrocheilus* is considered to be natural and to have probably existed for at least several hundred years.

INCOMPLETE ISOLATING MECHANISMS

(*a*) *Temporal isolation.*— Temporal isolation is ineffective in Cluculz River and in all inlets examined. The period of overlap of fully ripe individuals exceeds the period when only conspecific matings could occur. Apparently, both *C. commersonii* and *C. macrocheilus* begin their migration at about 6–9 C and spawn at about 10–12 C. *Catostomus commersonii* in eastern North America (Dence, 1948; Raney and Webster, 1942; Tremblay, 1962) and *C. macrocheilus* in Idaho (Keating, 1958) have similar temperature responses.

(*b*) *Habitat isolation.*— No habitat isolation occurs in the inlets. This is not surprising since these streams have few distinctive areas which could permit significant segregation in such large fish. Cluculz River has a diversified environment; species segregation there is probably explained by differences in habitat preference, rather than by exclusion of one species from mutually preferred areas.

(*c*) *Ethological isolation.*— The incomplete nature of the other potential isolating mechanisms, and the low number of hybrids compared with parental types, suggests that in *C. commersonii* and *C. macrocheilus*, as in most species (Mayr, 1963), ethological barriers are of major importance. Evidence from mate preference tests is lacking, but there is a basis for species recognition

based on size, morphology, and color. There is, however, no direct evidence that any of these physical differences are used for species recognition. Males in Cluculz River were often observed to move quickly downstream several meters or more and court females which had just moved off from shore to midstream. This suggested that auditory stimuli may be involved.

Contact between mating males and females is short, with no elaborate courtship (Reighard, 1920; present observations). Mating is promiscuous, with several males to each female. In both species, females generally move out from the river bank when ready to spawn and are pursued by several males. Even if females tended to pair with conspecific males, the opportunity for a male of the opposite species to enter the fertilization act with the other males does exist. Indeed, in several matings in area 6 which involved mainly *C. macrocheilus*, small males (probably *C. commersonii*) entered the spawning act along with larger *C. macrocheilus* males.

(*d*) *Hybrid embryo mortality.*— There is no evidence that interspecific crosses are less successful than conspecific crosses. It is considered unlikely that interspecific crosses using allopatric populations would have been less successful than those obtained by taking donors from an area of hybridization, since there is no reason to believe introgression is occurring (see later) and the donors are typical of their species.

(*e*) *Hybrid inviability and sterility.*— As in many species, *C. commersonii* and *C. macrocheilus* appear capable of producing large numbers of viable hybrids with no evidence of any effective postmating isolating mechanism. Differences in viability between hybrids and the parental species, however, could exist and be biologically significant. Sibley (1961) feels that hybrids between closely related species may be selected against by their intermediate characters being ill adaptive and reducing the chance for finding a mate.

Presumed female hybrids can be backcrossed at least to *C. commersonii*. There is no evidence that presumed hybrid males are fertile, but no difference from the parental species could be detected by examining slide preparations of gonads. Hubbs and Hubbs (1933), Hubbs and Drewry (1959), and Hubbs and Laritz (1961) have found sterile hybrids with abnormal meiotic segregation in the testes. Similar disorders could have existed in the sucker hybrids. Presumed male hybrids did produce milt as readily as the parental species.

The sex ratio of hybrids was similar to that of the parental species. Hubbs and Miller (1953) also reported a normal sex ratio in other sucker hybrids.

(*f*) *Interaction of isolating mechanisms.*— An interaction between habitat isolation and temporal isolation in Cluculz River in some cases produces effective segregation. Interspecific encounters must be very frequent in all streams. An interaction of temporal, habitat, and postmating isolating mechanisms is not considered sufficient to prevent swamping. The fact that numerous artificial crosses were made using suckers caught within a distance of 25 m indicates the lack of an effective interaction of isolating mechanisms, other than ethological isolation. Mate selection would therefore appear to be

of major importance. In each locality the percentage of F_1's, which probably constitute the majority of hybrids, is a crude measure of the effectiveness of isolation.

Effect of Hybridization

Sibley (1957) described the possible effects when an extrinsic barrier is broken. If isolating mechanisms have not been fully established, hybridization will result. The effects of hybridization vary depending on whether the hybrids are (i) at a selective advantage (resulting in swamping of the parental species, or perhaps introgression and change of the parental species to new adaptive peaks if only part of the hybrid gene complex is at an advantage); (ii) at a selective disadvantage (resulting in reinforcement and establishment of complete isolating mechanisms between the parental species). In relatively recent overlaps the direction of selection may be difficult to determine.

Selective Advantage of Hybrids

Since hybridization is not attributable to man's activities, hybrids have probably been produced since the start of sympatry, at least several hundred years ago. If hybrids, presently constituting on the average of 7% of the sucker population, were at a selective advantage, then fusion of the parental species should have occurred. Certainly, because of its self-accelerating nature, it would have gone to completion in a few localities.

There was no evidence for introgression (regarded here as the incorporation of genes of one species into another species through backcrossing and natural selection on the recombinants). Sympatric and allopatric populations were similar in variability, and the means of all characters in sympatric populations fell within the range of geographic variation of allopatric populations. It could be argued that *C. commersonii* in Burns Creek are products of introgression using interpretations similar to those of Hubbs (1961, p. 13–14) and Stebbins (1959). In this locality the species had a higher gillraker count (25–28) than had other sympatric populations (22–27). It seems more plausible that the high gillraker number was selected for in this creek without introgression. High scale counts for *C. commersonii*, resembling those of *C. macrocheilus*, occur well within the area of allopatry as well as in sympatry and are evidently not attributable to hybridization. An apparent closer resemblance in spawning coloration in the zone of sympatry than allopatry is also attributed to geographic variation. The resemblance has affected only the red band in spawning males. Brown and Wilson (1956) and White and Key (1957) have shown that morphological evidence must be used with caution and that it may be unreliable in showing introgression. It is concluded that swamping is evidently not occurring in the suckers, and that therefore reproduction in hybrids is selected against.

Selective Disadvantage of Hybrids

Selection against reproduction of hybrids may be effective in preventing swamping but not be detectable from laboratory observations. The effects

of selection against hybrids may sometimes be detected by examining the parental species for evidence of reinforcement. Reinforcement occurs if selection acts against hybrids, thereby promoting conspecific matings, until the isolating mechanisms have been perfected. Variability in the parental species which permits hybridization must be selected against. The intensity of selection determines the speed and extent of reinforcement. Sibley (1961) reviews the evidence in *Parus* where in the past 100 years hybridization has become reduced as a result of reinforcement. However, Hubbs (1961) reports mummified fish hybrids of several hundred years age from a population which presently has hybrids but where the parental species are still distinct. Also, hybrids may be at a selective advantage in some areas but at a disadvantage in others (Mayr, 1942, p. 265–266).

Characters affected by selection will be those which govern the meeting of different species. Reinforcement has been suggested for all isolating mechanisms, but the most convincing evidence exists for premating mechanisms. It is difficult to explain reinforcement of postmating mechanisms by the direct effects of selection. Indeed, it is difficult to separate the effects of reinforcement from geographic variation spuriously correlated with allopatry and sympatry (Hubbs and Strawn, 1957a; Mecham, 1961, p. 45).

In *C. commersonii* and *C. macrocheilus* no evidence was found of differences in spawning time or habitat between the zones of sympatry and allopatry. Of the characters examined which were considered to provide a possible basis for ethological isolation, no displacement was noted. The inability to demonstrate hybrid embryo mortality and hybrid sterility thus leaves no evidence of reinforcement in the investigated isolating mechanisms. From the beginning of sympatry, selection has not been severe enough to have prevented hybridization in any known locality. This is not altogether unexpected from the postulated cause of hybridization. Males are capable of fertilizing the eggs of many females and individuals of both sexes usually spawn in successive years. A mis-mated male or female has much opportunity in contributing to its own species through later conspecific mating. Selection against such an individual would be slow.

The lack of evidence for reinforcement of isolating mechanisms in *C. commersonii* and *C. macrocheilus* suggests that the mechanisms present were developed in allopatry. As such, the mechanisms would be incidental by-products of divergence arising through selective pressures unrelated to reproductive isolation. This is generally regarded to be the origin of isolating mechanisms by Mayr (1942, 1959, 1963), Mecham (1961), Moore (1957), and Muller (1942). However, Dobzhansky (1940, 1951, p. 207–211) suggests that reinforcement is generally necessary to complete speciation and fully establish isolating mechanisms. It is possible that some mechanisms arose through reinforcement with other species. *Catostomus commersonii* was probably sympatric with *C. catostomus* (with which it hybridizes in one area of introduction (Hubbs et al., 1943)) and several related species during the last glaciation. *Catostomus macrocheilus* was sympatric with several species of *Catostomus*

in its Columbia refugium and hybridizes with *C. columbianus* (Hubbs et al., 1943; author's work). Koehn (MS, 1967) and Smith (1966) have found evidence of introgressive hybridization in *Catostomus* and believe hybridization may have been important in the evolution of some populations.

From the apparent time during which hybridization has occurred and evidence that swamping has not occurred, it is concluded that there are selective pressures preventing hybrids from contributing to the gene pool of the parental species. Slow selection against participants of hybridization would account for the apparent absence of reinforcement of the isolating mechanisms at the present stage. In agreement with the contention of Mayr (1963) that in vertebrates there is little evidence that hybridization has contributed to speciation, there is no evidence here that hybridization has had any effect in the speciation of *C. commersonii* and *C. macrocheilus*.

CONCLUSIONS

Catostomus commersonii and *C. macrocheilus* have been in secondary contact in some areas for at least several hundred years. Presumed F_1 hybrids made up an average of 7% of the individuals of the combined populations in the nine lakes where hybrids were found.

Hybridization is occurring in undisturbed areas and could not be attributed to the environmental factors frequently invoked as conducive to hybridization in fish. There was no evidence of backcrossing or introgression, nor of reinforcement of the isolating mechanisms. An interaction of temporal, habitat, and several investigated postmating isolating mechanisms is not considered sufficient to explain the absence of swamping. Ethological isolation is inferred to keep F_1 hybrids rare, whereas unknown factors prevent swamping by selection against backcrosses.

Accidental fertilization may account for some hybrids, but the main cause of hybridization is considered to be males of one species entering the spawning act with males and a female of the other species. Since mis-mated males can contribute to conspecific matings at other times, selection against such individuals would be slow. This is in agreement with the contention that hybridization has probably been occurring since the beginning of sympatry although there is no evidence of reinforcement. Effective isolating mechanisms were established in allopatry and a slow process of reinforcement is probably occurring.

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Hematological Changes in Coho Salmon Fed a Folic Acid Deficient Diet[1]

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ABSTRACT

Coho salmon (*Oncorhynchus kisutch*) fed a diet deficient in folic acid for 24 weeks developed megaloblastic, normochromic macrocytic anemia. The anemia was further characrerized by the presence of poikilocytic erythrocytes which were observed in stained preparations of peripheral blood. These abnormally shaped cells appear to be an important aid in the identification of folic acid deficiency. The anemia disappeared when the deficient fish were fed a recovery diet containing folic acid for 8 weeks.

INTRODUCTION

In previous experiments on the vitamin requirements of salmonids, folic acid deficiency was shown to cause dietary anemia (McLaren et al., 1947; Wolf, 1951; Halver, 1957; Phillips et al., 1961, 1962; Phillips, 1963). In these experiments parameters used to measure hematological changes were restricted to either the red blood cell (RBC) count, hemoglobin determination, or a combination of the two. However, the type of anemia that developed from folic acid deficiency was not described.

The intent of this report is: (1) to describe the type of anemia that developed in coho salmon (*Oncorhynchus kisutch*) fed a diet deficient in folic acid; and (2) to emphasize the importance of examining blood smears and utilizing red blood cell indices to aid in the identification of the type of anemia.

METHODS

During routine hematology at the termination of an experiment of the Western Fish Nutrition Laboratory to determine the necessity of adding vitamin supplement to the Oregon Moist Pellet (OMP) (Hublou et al., 1959), an abnormal blood picture was detected in fish fed a diet deficient in folic acid. The unusual morphology of certain cells prompted continuation of that particular phase of the original study. The experiment was conducted on coho salmon at the Hagerman Field Station, Hagerman, Idaho, where the water temperature was constant at 15 C. The test diet (W. E. Shanks, unpublished data) was compounded to contain 12.5% of unsupplemented OMP, 86.5% of vitamin-free basal ration and 1% of vitamin pack similar to the regular OMP supplement except for the lack of folic acid. Thus, the only source of folic acid was that present in the natural ingredients contained in the 12.5 g

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OMP/100 g total diet. Control fish were fed a complete test diet (Halver, 1957) containing twice the normal amount of water soluble vitamins. Fish were fed twice daily, 6 days weekly.

After feeding the deficient diet for 24 weeks, growth was depressed and mortality had begun to occur. At the end of this 24-week period, hematological examinations were performed on 40 folic acid deficient fish with an average weight of 16 g, and 40 control fish with an average weight of 20 grams. Two weeks later the remaining fish were divided into the following groups of 60 fish each and fed as indicated: (1) control fish fed complete test diet; (2) deficient fish continued on the folic acid deficient diet; and (3) deficient fish placed on recovery and fed the same diet as control fish. Because of the apparent slow rate of recovery in group 3, at the end of 4 weeks the amount of folic acid was increased to five times that of the control group. The recovery study was terminated at the end of 8 weeks.

Hematological methods used were those described by Hesser (1960). Hemoglobin concentrations were determined by the cyanmethemoglobin method.[2] Peripheral blood smears and imprints of hemopoietic kidney were prepared (Ashley and Smith, 1963), fixed in methyl alcohol, and stained by the following method (G. W. Klontz, personal communication): 1, Leishman stain (stock solution) — 3–4 min; 2, Giemsa (3.5 ml stock solution to 50 ml distilled H_2O buffered to *p*H 6.4) — 5–7 min; 3, agitate in distilled water — 2 min; 4, rinse in running tap water and air dry. Stock stains were prepared by the method of Jones (1961).

Readily determined erythrocytic indices furnishing information on the average volume and hemoglobin content of red blood cells were computed for each group (Seiverd, 1960).

Significant differences were established at the .01 level by the Student *t*-Test.

RESULTS

Hematological values of controls and folic acid deficient salmon prior to recovery diet treatment are listed in Table I. Variations in RBC counts of the deficient group were extensive. Some fish had values similar to those of controls while others showed severe anemia. In general, RBC count, volume of packed cells, and hemoglobin were all depressed in the deficient fish; however, only the RBC counts were significantly lowered. Mean cell volume and mean corpuscular hemoglobin were significantly higher in the folic acid deficient fish than the control group.

Examination of peripheral blood smears from the deficient fish revealed erythrocytic poikilocytosis and anisocytosis (Fig. 1–3). RBC nuclei were often segmented into small lobes (Fig. 4). Imprints of hemopoietic kidney revealed hyperplasia and hypertrophy of erythroid elements (Fig. 5 and 6).

[2]Hycel, Inc., Houston, Texas.

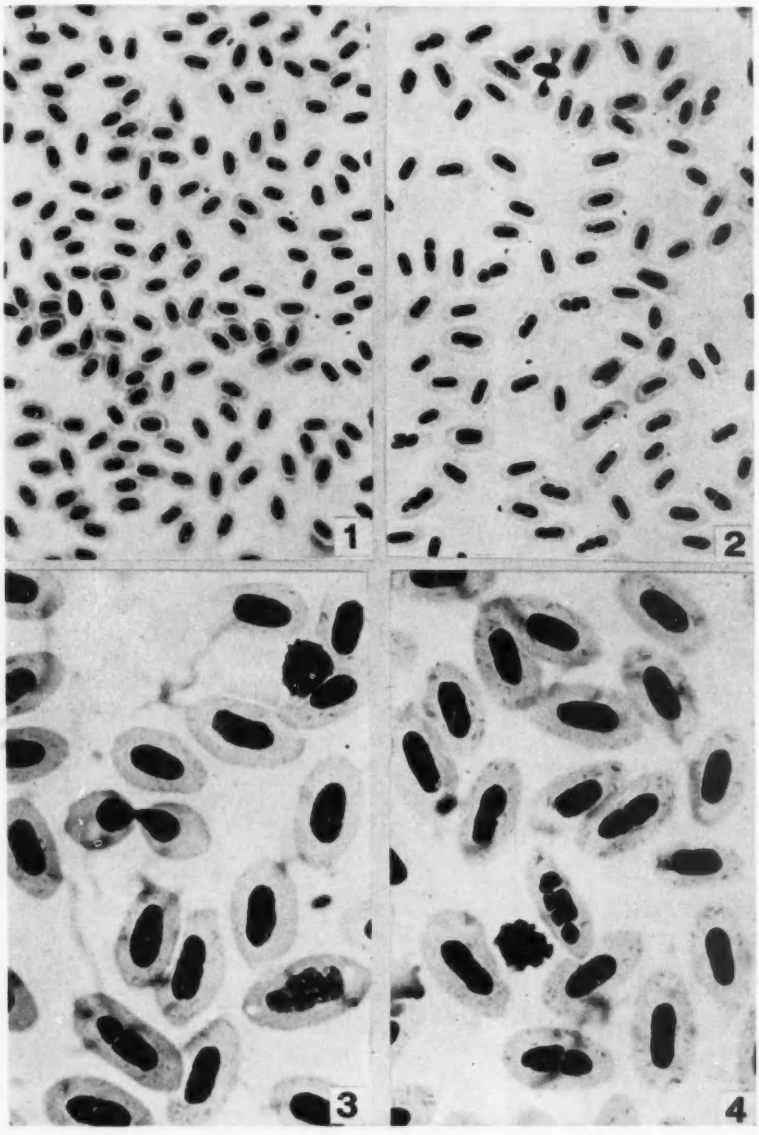

FIG. 1. Normal erythrocytes. Control coho salmon. ×357.

FIG. 2. Anisocytosis, poikilocytosis, and segmentation of nuclei of erythrocytes from folic acid deficient coho salmon. ×357.

FIG. 3. Poikilocytic erythrocyte from folic acid deficient coho salmon in center of field. ×714.

FIG. 4. Segmented nuclei in erythrocytes from folic acid deficient coho salmon. ×714.

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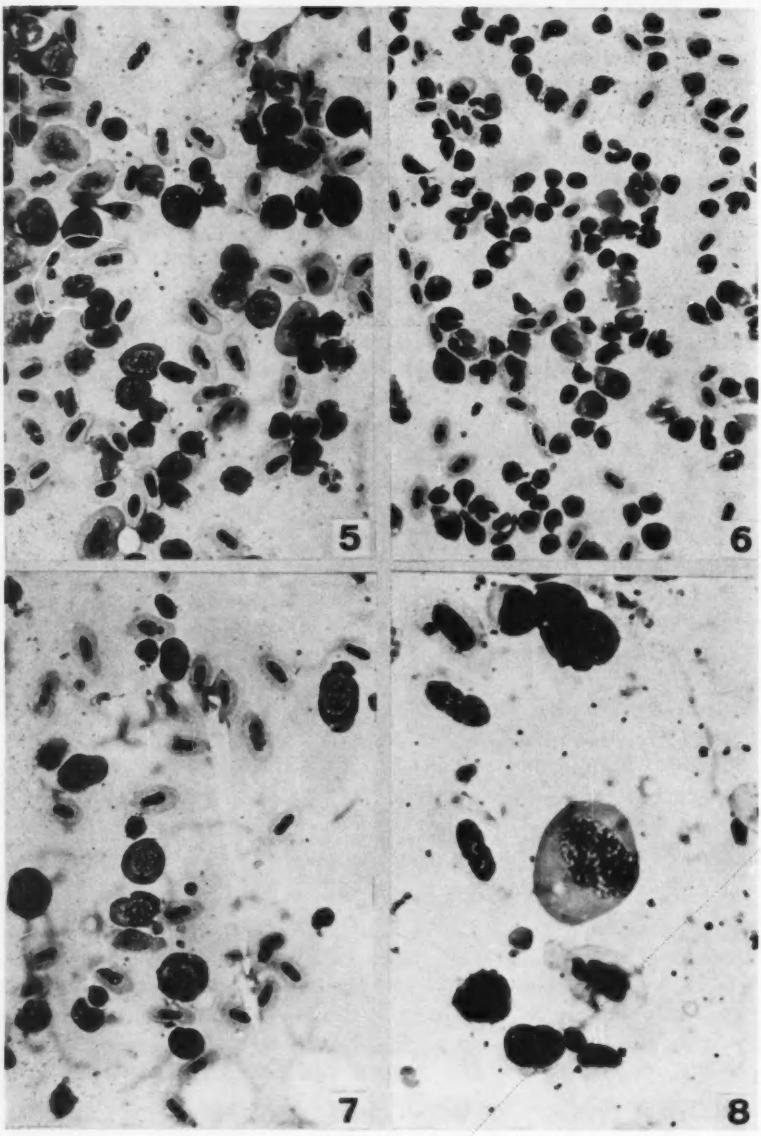

FIG. 5. Erythroid hyperplasia and hypertrophy in folic acid deficient coho salmon. Kidney imprint. ×357.

FIG. 6. Apparently normal erythropoiesis. Control coho salmon. Kidney imprint. ×357.

FIG. 7. Increased interchromatin spaces in nuclei of megaloblastic erythroid elements from folic acid deficient coho salmon. Kidney imprint. ×357.

FIG. 8. Eccentric somewhat lobulated nucleus in immature erythrocyte from folic acid deficient coho salmon. ×714.

TABLE I. Average hematological values of control and folic acid deficient coho salmon prior to placing on recovery.

	Controls	Deficient
Red cell count	1,360,000	920,000
(erythrocytes/mm^3)	(920,000–1,740,000)[a]	(520,000–1,410,00)[a]
Hemoglobin	8.73	7.63
(g/100 ml)	(6.9–10.6)	(5.3–10.3)
Hematocrit	45	38
(% packed cell volume)	(36–60)	(26–56)
MCV[b]	332	433
(μ^3)	(252–458)	(229–653)
MCH[c]	65.2	86.0
($\mu\mu$g)	(48.4–80.9)	(52.7–119.2)
MCHC[d]	19.5	20.2
(% hemoglobin/packed cell volume)	(13.5–25.2)	(13.0–26.9)

[a]Range.
[b]Mean cell volume.
[c]Mean corpuscular hemoglobin.
[d]Mean corpuscular hemoglobin concentration.

Many of these cells resembled the megaloblasts of higher vertebrates (Reisner, 1958; Wheby, 1966). There was an increase in both number and size of erythroid elements, increased interchromatin spaces (Fig. 7) and more immaturity of nuclei in comparison with cytoplasm. In addition, the nuclei of some cells were eccentric (Fig. 8), and in others breaking of chromatin was noted.

At the termination of the experiment, blood values of the recovery group were slightly elevated above those of the control group (Table II). Values of the deficient fish remained similar to those recorded prior to placing them on recovery.

Examination of peripheral blood smears revealed that poikilocytic erythrocytes, still common in the deficient group, were absent or present only in very small numbers in the majority of smears examined from the recovered fish. In imprints of hemopoietic kidney from recovered fish, a lack of megaloblastic erythroid precursor cells and reduced erythroid hyperplasia were apparent whereas both were still common in the folic acid deficient fish.

DISCUSSIONS

Anemia which developed in coho salmon fed the folic acid deficient diet was apparently of the megaloblastic, normochromic macrocytic type. These characteristics agree with results observed in other animals fed diets deficient in folic acid (Hogan and Parrott, 1940; Campbell et al., 1945; Cartwright et al., 1949, 1950; DaSilva et al., 1955; Slungaard and Higgins, 1956; Dinning, 1962).

TABLE II. Average hematological values of control, folic acid deficient, and recovery coho salmon at termination of experiment.

	Controls	Deficient	Recovery
Red cell count (erythrocytes/ mm^3)	1,320,000 (950,000–1,780,000)[a]	959,000 (580,000–1,170,000)[a]	1,330,000 (1,080,000–1,800,000)[a]
Hemoglobin (g/100 ml)	8.2 (6.8–9.7)	7.7 (5.7–9.4)	8.9 (7.3–10.6)
Hematocrit (% packed cell volume)	44.5 (27–59)	41.2 (31–55)	48.0 (37–52)
MCV[b] (μ^3)	339 (257–466)	440 (321–793)	360 (267–471)
MCH[c] ($\mu\mu g$)	61.9 (47.2–75.1)	82.3 (51.0–134.4)	67.5 (52.5–86.8)
MCHC[d] (% hemoglobin/ packed cell volume)	18.3 (14.2–21.7)	18.6 (15.8–21.6)	18.6 (16.2–21.5)

[a]Range.
[b,c,d]See footnotes b, c, and d to Table I.

Although the RBC count in some deficient fish fell within the range of the controls, poikilocytosis was apparent in peripheral blood smears from several of these deficient fish. Previous investigators of folic acid deficiency in fish did not mention having observed poikilocytic RBC's. However, personal communications revealed that such cells were observed while doing RBC counts in folic acid deficient brook trout and chinook salmon (A. M. Phillips, Jr. and J. E. Halver, personal communications).

Campbell et al. (1945) reported a macrocytic normochromic anemia in chicks fed a diet deficient in vitamin B_c (folic acid). They noted increased numbers of erythroid normoblasts and pronormoblasts in peripheral blood, poikilocytosis, a moderate to marked anisocytosis, and the breaking of nuclear chromatin in immature erythroid cells.

Macrocytosis and aberrant RBC nuclei may result from impairment of the folic acid dependent replication of nuclear deoxyribonucleic acid which must precede normal mitotic activity. In deficiency, erythroid precursor cells are unable to divide into normal smaller daughter cells; hence, these cells divide abnormally slowly and are finally released into the blood stream as large macrocytes (Jacob, 1966).

The hemoglobin portion of the cell was apparently unaffected as indicated by the mean corpuscular hemoglobin concentration remaining essentially the same while the mean corpuscular hemoglobin of the deficient group was elevated over that of controls. This indicated that the increase in hemoglobin

in macrocytic erythrocytes was parallel with, and not in excess of, the increase in cell volume.

Average hematological values of the deficient group were similar at the beginning and at the termination of the recovery period. It appeared that the 12.5% unsupplemented OMP present in the deficient diet supplied sufficient folic acid to partially satisfy the requirement, and that a large individual variation for the requirement of folic acid was evident within this particular group of fish.

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The Isopod *Mesidotea entomon* in the Chignik Lakes, Alaska[1]

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ABSTRACT

Studies on the isopod *Mesidotea entomon* (Linnaeus) from 1961 to 1964 in the two Chignik lakes, Alaska, showed that at least part of the population was benthic during daylight and pelagial at night. In Chignik Lake the isopod was most abundant in association with organic and mud bottoms and areas where salmon carcasses accumulate. Abundance decreased from early to late summer, perhaps because of a postreproductive mortality. It is suggested that males grow faster than females, though both sexes mature at age I+ and that growth terminates with maturation. Most reproduction apparently occurs in early summer but some ovigerous females were found as late as September. Length at maturity in both sexes was less than reported by other workers in Arctic marine populations. Mature isopods were smaller in the upper lake (males 40–46 mm, $\overline{X}$ = 43; and females 29–38 mm, $\overline{X}$ = 33) than in the lower lake (males 41–65, $\overline{X}$ = 55; and females 29–47 mm, $\overline{X}$ = 39) of the Chignik River system. Females in the upper lake had a higher average fecundity and a greater increase in fecundity per unit increase of length than those in the lower lake.

INTRODUCTION

The isopod *Mesidotea entomon* (Linnaeus) occurs commonly in the Chignik lakes, Alaska (Fig. 1). Numerous collections and observations were made of this isopod in the summers of 1961–64 during an investigation of the lacustrine life history of sockeye salmon, *Oncorhynchus nerka* (Walbaum) (Narver, MS, 1966). This paper is the first report on the lacustrine biology of *M. entomon*. It is usually described as a littoral marine species with circumpolar distribution (Richardson, 1905; Boone, 1920; MacGinitie, 1955; McCrimmon and Bray, 1962). In Arctic oceans of western North America it may prefer shallow, brackish water (MacGinitie, 1955; McCrimmon and Bray, 1962). Boone (1920) observed that some older individuals ascended creeks to the lakes along the Arctic coast near the Alaska–Yukon border. *Mesidotea entomon* occurs in six lakes in the central Swedish lowlands as well as in the brackish waters of the Baltic (Lockwood and Croghan, 1957). It has also been reported from four lakes on Victoria Island in the Canadian Arctic (Johnson, 1964).

The morphology of specimens of *M. entomon* from the Chignik lakes was compared with the descriptions of this species as given by Richardson

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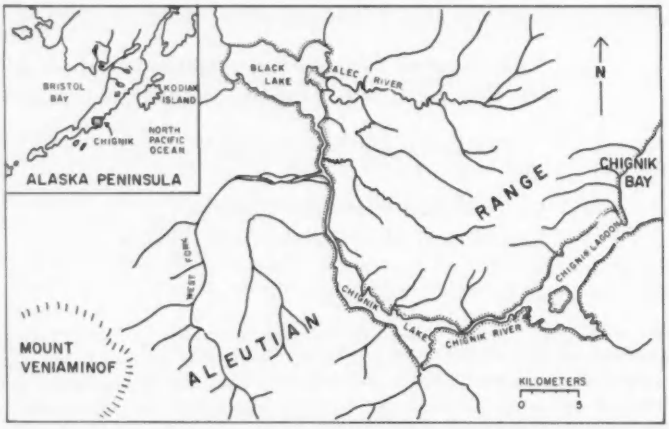

FIG. 1. Chignik River watershed with an inset of western Alaska.

(1905), Boone (1920), and McCrimmon and Bray (1962). No differences were observed except that the Chignik lakes population tended to be smaller in length than the marine populations. Specimens from Chignik Lake are in the invertebrate collection of the College of Fisheries, University of Washington, Seattle, Wash.

STUDY AREA AND METHODS

The Chignik lake system, consisting of two lakes, is located on the south side of the Alaska Peninsula about 270 km southwest of Kodiak Island (Fig. 1). Both lakes are completely freshwater and were described in detail by Narver (MS, 1966). Black Lake (the upper lake) has a maximum depth of 6 m, a surface area of 43 km^2, and an elevation of about 15 m. The bottom is composed mainly of sand and silt, with considerable organic detritus near the outlet and in the northeast corner. Chignik Lake has a maximum depth of 64 m, an area of 24 km^2, an elevation of about 5 m, and a rather precipitous shore. Its bottom is composed primarily of rubble and boulders with gravel, silt, and organic deposits interspersed. Chignik Lake drains into an estuarial lagoon.

The primary sampling device in both lakes was a tow net 5.2 m long with a 3.24-m^2 opening, towed in the top 4 m at an average speed of 1.4 m/sec by two boats powered by 40-hp outboard motors (Narver, MS, 1966). The tow net meshes were of knotless nylon and decreased in size from 2 cm^2 near the opening to 0.33 cm^2 at the cod end. The net was designed for sampling juvenile sockeye salmon in the pelagial zone. Since these fish exhibit diel movement in the summer months and occur near the surface of the lake only from dusk until midnight or a little later, most townetting was conducted at this time. The assumption was that within a lake the availability to the tow net of the

species of interest was constant. The catches were used to compare abundance between lakes as well as among statistical areas (four in Black Lake and five in Chignik Lake) and among dates within either lake. The catch from each 10-min tow was enumerated by species and all isopods were preserved, together with fish samples, in 10% formalin.

Analysis of variance techniques were used to test the hypothesis that the density of *M. entomon* in either lake was the same in all years, in all lake areas, and in two time periods (late July to early August and late August to early September). I previously discussed these tests in considering the reliability of tow net catches of fish in the Chignik lakes as an index of abundance (Narver, MS, 1966).

Mesidotea entomon was also collected coincidentally with fish in gill nets, bottom trawls, and fyke nets. Lightweight monofilament nylon gill nets (1.9–4.4 cm stretched mesh) were set on the bottom and fished all night on 26 occasions. A small otter trawl with a cod end of 0.6-cm stretched mesh was fished several times on the bottom during the day.

A total of 1599 isopods from Chignik Lake and 58 from Black Lake were sexed, classified as to maturity, and measured (as in McCrimmon and Bray, 1962).

Fecundity was determined for ovigerous females by counting eggs contained in the broad pouch. The relationships between fecundity and length of females in Chignik Lake and Black Lake were determined using techniques of analysis of covariance (Narver, MS, 1966).

ABUNDANCE AND DISTRIBUTION

The night tow net catches revealed that the relative abundance of the isopod was far greater in Chignik Lake than in Black Lake (Table I). From 1961 to 1964 this gear caught 5033 isopods in Chignik Lake (347 10-min tows) and 351 in Black Lake (174 tows). At least part of the isopod population in Chignik Lake exhibited diel movement, being benthic during daylight hours and pelagic at dark. No isopods were caught in daylight tows made in the top 4 m, whereas daylight trawl catches from mud bottoms were large. In the outlet bay of Chignik Lake *M. entomon* was observed moving on the bottom detritus during daylight in 1965 by divers (M. L. Dahlberg, personal communication). Density was estimated at 3–4 per m^2. (Bottom trawls were not made at night.) There is no diel movement in the related species *Idothea neglecta* (Kjennerud, 1950).

The tow net catches of isopods in Chignik Lake were significantly different among years (4), statistical areas (5), and time periods (2) (Table II). Interactions in the analysis of variance were not significant. The differences in catches among years presumably indicate fluctuations in year-class strength. The geometric mean catches per 10-min tow were lower at the upper end of Chignik Lake than at the lower, the means for the five statistical areas from inlet to outlet being: 9.1, 9.4, 13.2, 13.6, and 16.7. This might be related to environmental differences between the two ends of the lake. Each September

TABLE I. Geometric mean catches of *M. entomon* per 10-min net tow at night in the top 4 m in the 4 years of sampling. Years divided at August 15; mean catches of each area weighted by size of area; numbers of tows in parentheses.

Year	Chignik Lake		Black Lake	
	Before August 15	After August 15	Before August 15	After August 15
1961	19.8	15.9	0.9	0.5
	(42)	(41)	(13)	(10)
1962	21.7	6.8	0.1	0.5
	(28)	(75)	(16)	(45)
1963	23.5	8.1	1.2	2.6
	(14)	(56)	(14)	(7)
1964	14.9	5.9	2.2	8.6
	(16)	(44)	(12)	(13)

TABLE II. Results of three-way analysis of variance for Chignik Lake mean tow net catches of *M. entomon*.

Source	df	F ratio
Year	3,276	3.37147*a*
Area	4,276	4.42561**
Time	1,276	21.49831**
Year × area	12,276	1.24893
Year × time	3,276	1.77837
Area × time	4,276	1.94137
Year × area × time	12,276	0.94158

a $P<0.05$ but >0.01; ** $P<0.01$.

and October 100–200 thousand sockeye salmon carcasses are present in the lower end of the lake, whereas few are found at the upper end. Also there are extensive bottom deposits of organic material in the area near the lake outlet; McCrimmon and Bray (1962) found the density of *M. entomon* was highest in mud bottom areas and lowest in sand and gravel. Furthermore, the water in the upper end of the lake is often more turbid from the runoff of glaciers than the water in the lower end. The decrease in abundance between midsummer and late summer (Table I) could be a result of postreproductive mortality. Exoskeletons of adult isopods are common on most beaches of Chignik Lake in late summer. However, no special effort was made to collect them. *Idothea neglecta* breeds once only before it dies in late summer at age I (Kjennerud, 1950). McCrimmon and Bray (1962) believe that most individuals of *M. entomon* die after their first reproductive season.

The tow net catches of isopods in Black Lake were not significantly different among lake areas, sampling times, or years. However, isopod catches were low in Black Lake.

No information was obtained on horizontal movement, but such movement is possible since the animal is extremely mobile both in swimming and in crawling over the bottom.

Bottom gill-net catches at night in Chignik Lake suggest that *M. entomon* may increase in abundance with depth:

Depth (*m*)	0–10	11–20	21–30	31–40	41–50	>50
No. of sets	15	3	4	1	0	3
Geometric mean catch	8.9	20.8	33.2	70.0	–	48.3

However, the largest tow net catches in Chignik Lake were in the shallowest area near the outlet.

Fyke nets, set in the Chignik River at the outlet of the lake at night in May and June, caught small numbers of isopods.

A bottom trawl was fished only in daylight on flat bottoms of gravel, silt, and organic composition. The largest trawl catches of isopods were made in an area of Chignik Lake heavily used by spawning sockeye salmon. Skeletal remains of salmon were common in the bottom material collected in trawls that had large numbers of isopods.

Mesidotea sp. (presumably *entomon*) occurs in several of the large lakes on the northern side of the Alaska Peninsula according to biologists familiar with the region. All of these lakes are close to tidewater and have a low elevation. The isopod is common in Becharof and Ugashik lakes northeast of the Chignik watershed, especially in catches taken by fyke nets in May during sampling of seaward-migrating sockeye salmon (Dr C. J. DiConstanzo, personal communication). This isopod may occur in the Naknek system (Dr W. L. Hartman, personal communication), but has not been recorded in the Kvichak or Nushagak systems of Bristol Bay, Alaska (Mr O. J. Kerns, personal communication).

The populations of *M. entomon* in western Alaskan lakes may have arisen from postglacial invasions from the sea; it certainly occurs in Chignik Lagoon. A direct postglacial invasion was suggested for freshwater lakes in Sweden (Lockwood and Croghan, 1957). Alternatively, like those in lakes on Victoria Island in the Canadian Arctic (Johnson, 1964), the populations may be marine-glacial relics.

FOOD HABITS

Most aquatic isopods are omnivorous scavengers feeding on dead animals such as fish, detritus, and other organic material. In Chignik Lake it was common for *M. entomon* to reduce a 100- to 200-mm pygmy whitefish, *Prosopium coulteri* (Eigenmann and Eigenmann), to a skeleton during a 10-hr gill-net set. Divers in 1966 found an aggregation of 10–15 isopods feeding on a dead sculpin on the bottom during the day (M. L. Dahlberg, personal

communication); from their trails left in the detritus, *M. entomon* appeared to the divers to wander randomly over the bottom. *Mesidotea entomon* probably feeds extensively on sockeye salmon carcasses. Kjennerud (1950) reported that dead fish held in seawater prior to marketing were damaged by the isopod *I. neglecta*. *Mesidotea entomon* was reported by McCrimmon and Bray (1962) to feed on animal remains including euphausiids.

In addition to being a scavenger, *M. entomon* apparently feeds on zooplankton in the pelagial zone at night in Chignik Lake. Of 20 isopods (3 mature ♂, 2 immature ♂, 7 immature ♀, and 8 spent ♀) captured by tow net on August 25, 1962 in Chignik Lake, 8 had empty stomachs and 11 had broken-up copepods. Five of the empty stomachs occurred among the immature females. Other items found in the stomachs were cladocern remains, "silt," diatoms, and juvenile sockeye scales. The last presumably were injested during the netting.

PREDATION

There are few fish in the lakes capable of feeding on the larger isopods during the summer although juvenile coho salmon, *Oncorhynchus kisutch* (Walbaum), take a few of the smaller isopods. A large population of Dolly Varden, *Salvelinus malma* (Walbaum), probably winters in Chignik Lake (Narver and Dahlberg, 1965) and isopods may be a winter food.

AGE AND GROWTH

To obtain a definitive concept of age and growth of *M. entomon*, one must either have samples throughout the year or establish an aquarium population. Neither was established for *M. entomon* in the Chignik lakes, but a series of samples (1454 specimens from Chignik Lake and 50 from Black Lake) were obtained during the months of June through September. Analysis of these permits one to draw some tentative conclusions.

The range in length for isopods from Chignik Lake was 11–65 mm and for Black Lake 22–46 mm. The average length for mature males and females in Chignik Lake was 55 and 39 mm and in Black Lake it was 43 and 33 mm.

Although 11 rather large samples of *M. entomon* from Chignik Lake (Fig. 2) were used, it was difficult to obtain a clear idea of age and growth. Age interpretation is based on growth. If the rates of growth were similar for the two sexes and growth continued to death, then it would appear that Chignik Lake males mature 1 year later than females (Fig. 2). However, the available evidence is contrary to this. In three species of marine isopods males grow faster than females, females cease to grow after reaching maturity, and maturity occurs in their second year of life (age I) (Kjennerud, 1950: *Idothea neglecta*; Naylor, 1955: *I. emarginata*; McCrimmon and Bray, 1962: *M. entomon*). Such sexual differences in the patterns of growth are suggested for the isopod in the Chignik lakes from the narrower length range for females than for males and lack of modal progression (Fig. 2).

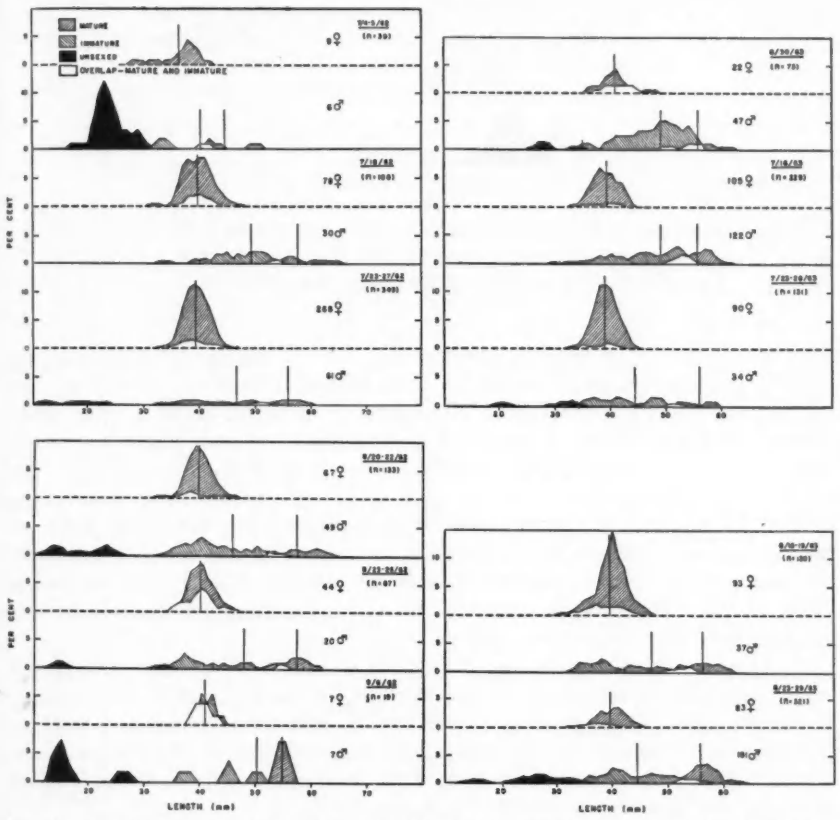

FIG. 2. Length frequencies of males and females of *M. entomon* in samples (corresponding to Table III) from Chignik Lake in 1962 and 1963 according to stage of maturity; data smoothed by threes and expressed in per cent of total sample size. The vertical lines indicate the sample means for all females, all males, and mature males.

The mean lengths of females and mature males from Chignik Lake each varied little through the summers of 1962 and 1963 (Fig. 2) so there is little to indicate growth. The data for Black Lake samples (Fig. 3) show a similar situation, but both males and females were considerably smaller than in the lower lake.

SEXUAL MATURITY AND FECUNDITY

MATURITY

Mature individuals of both sexes (McCrimmon and Bray, 1962) were found in Chignik Lake as early as June 30 and as late as September 6 (Table

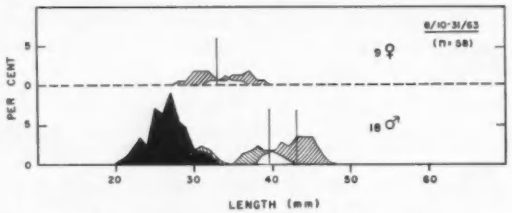

FIG. 3. Length frequencies of males and females of *M. entomon* in samples (corresponding to Table III) from Black Lake in 1963 according to stage of maturity. Interpretation as in Fig. 2.

III). The majority of females were mature. Of the mature females, a rather small portion bore ova or larvae. In both 1962 and 1963 an average of over 92% of the mature females were in the postreproductive stage (empty brood pouch). The percentage in postreproductive condition was smallest in early and late summer. Presumably breeding (described by Kjennerud, 1955) and larval development occurred mainly in early spring, and many of the females in the postreproductive stage died by early September. However, some reproduction extended to September since a few ovigerous females were still present. Mature males constituted considerably less than half of the total males in the Chignik Lake samples and the percentage was highest in late August and early September (Table III).

In western Arctic Ocean populations of *M. entomon* reproduction appears to be continuous throughout the summer (MacGinitie, 1955; McCrimmon and Bray, 1962). The reproductive season is mainly in the spring for *I. neglecta* (Kjennerud, 1950) and *I. emarginata* (Naylor, 1955). However, Naylor also suggests that each year-class may produce two broods, one in the fall of its first year and another the following spring.

The population structure of *M. entomon* in Black Lake appears considerably different from that in Chignik Lake. The percentages of mature males and of ovigerous females in the limited samples taken (Table III) were much higher than in the Chignik Lake population.

Mesidotea entomon matures at a smaller size in the Chignik lakes than in at least one Arctic marine population. In Chignik Lake mature males were from 41 to 65 mm ($\overline{X}$ = 55) and mature females were from 29 to 47 mm (39). In Black Lake the isopod matured at an even smaller size; males were from 40 to 46 mm (43) and females from 29 to 38 mm (33). McCrimmon and Bray (1962) reported that mature males ranged from 65 to 95 mm, females from 50 to 79 mm.

FECUNDITY AND DEVELOPMENT

The results of analysis of covariance indicated that Black Lake females had higher mean fecundity and a greater increase in fecundity per unit increase of length than Chignik Lake females, so the data were best represented

TABLE III. Numbers of immature and mature males and females in the 1962 and 1963 tow net samples of *M. entomon* from the Chignik lakes.

Date	Males				Females			
	Unsexed	Immature	Mature (% of ♂)	Total	Immature	Mature (% of ♀)	Ovigerous (% of mature ♀)	Total
Chignik Lake, 1962								
July 4–5	24	3	3 (50.0)	6	1	8 (88.9)	2 (25.0)	9
July 18	0	23	7 (23.3)	30	11	67 (85.9)	6 (8.9)	78
July 23–27	20	41	20 (32.8)	61	34	234 (87.3)	2 (.6)	268
Aug. 20–22	17	37	12 (24.5)	49	6	61 (91.0)	3 (4.9)	67
Aug. 25–26	3	13	7 (35.0)	20	14	30 (68.2)	6 (20.0)	44
Sept. 6	5	3	4 (57.1)	7	4	3 (42.8)	1 (33.3)	7
Total	69	120	53 (30.6)	173	70	403 (85.2)	20 (4.9)	473
Chignik Lake, 1963								
June 30	4	40	6 (13.0)	47	7	15 (68.2)	9 (60.0)	22
July 16 (trawl)	2	81	41 (33.6)	122	9	96 (91.4)	6 (6.2)	105
July 23–28	7	29	5 (14.7)	34	8	82 (91.1)	2 (2.4)	90
Aug. 15–19	0	24	13 (35.1)	37	14	79 (84.9)	3 (3.8)	93
Aug. 23–29	57	106	75 (41.4)	181	14	69 (83.1)	7 (10.1)	83
Total	70	280	140 (33.3)	421	52	341 (86.7)	27 (7.9)	393
Black Lake, 1962 and 1963								
Aug. 27/62	6	1	1 (50.0)	2	0	0	0	0
Aug. 10/63	6	6	6	12	1	5 (83.3)	3 (60.0)	6
Aug. 30–31/63	15	3	5 (62.5)	8	1	2 (66.6)	2 (100.0)	3
Total	27	10	12 (54.5)	22	2	7 (79.1)	5 (71.4)	9

by a single regression line for each lake (Fig. 4). This interpretation should be accepted with caution because of the scarcity of Black Lake data. The regression equation for the Chignik Lake data for 1962 and 1963 was $Y = 158.5 + 8.41 (X - 39.7)$ and that for the Black Lake data for 1963 was $Y = 179.3 + 14.00 (X - 34.0)$.

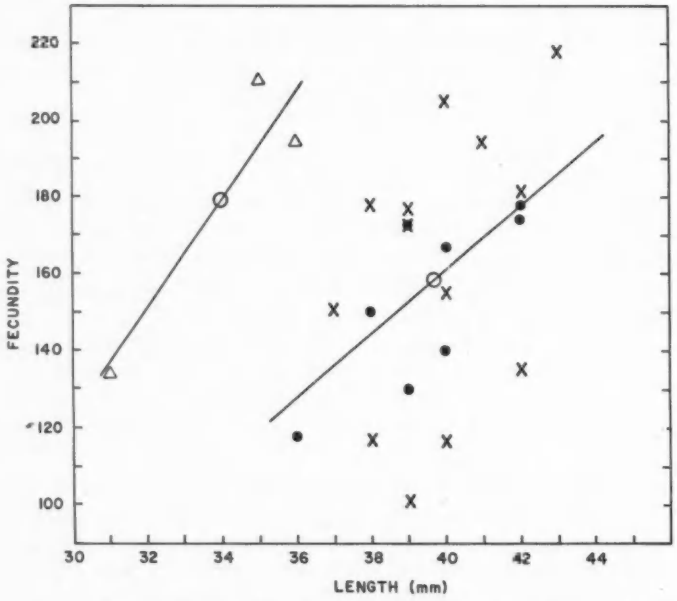

FIG. 4. Relationships between fecundity and length in the female of *M. entomon* from the Chignik lakes. Plotted points represent single females: ● = 1962 Chignik Lake, × = 1963 Chignik Lake, △ = 1963 Black Lake, and ○ = mean.

There may be considerable loss of embryos from the brood pouch during development from ova to larvae. The average count of ova in apparently undisturbed brood pouches from Chignik Lake females in 1962 and 1963 was much greater (158.6) than the average count of larvae (91.8). Such a suggested loss is reasonable since the average size (longest diameter) of the eggs was 1.17 mm (range 1.0–1.5) whereas the average length of the larvae prior to release was 3.9 mm (3.1–4.5). (The greatest length of early-stage *M. entomon* eggs was reported as 1.08 mm by MacGinitie (1955) whereas newly deposited larvae were reported as 3–5 mm in length by McCrimmon and Bray (1962).) A smaller number of larvae than of ova in brood pouches was reported by Jancke (1926, cited in Kjennrud, 1950) in the related species *I. viridis*, but not by Kjennerud in *I. neglecta* nor Naylor (1955) in *I. emarginata*.

The number of larvae carried by female *M. entomon* of the Arctic marine population studied by MacGinitie (1955) in mid-July ranged from 482 to 776 as compared with an average for the Chignik Lake population of 91.8. The largest female was 74 mm (no range given), whereas the largest female from Chignik Lake was 43 mm.

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NOTES

Pesticide Residue in Freshwater Fish Oils and Meals

The large scale use of pesticides for industrial and agricultural purposes can result in reduction in wildlife populations such as birds or fish (Bernard, 1963; Kerswill, 1958). The long-range problems associated with the use of pesticides as they affect biological systems are well reviewed by Rudd (1964). Fish in general, and carnivorous fish in particular, have the capacity to concentrate and store pesticides, without themselves being outwardly affected, except when the concentration of pesticides in their body reaches fairly high levels (Hickey et al., 1966).

The hazard to human beings from eating fish containing pesticides is remote since the pesticide residues are usually concentrated in the viscera (Rudd, 1964). However, fish-eating mammals and especially birds can be seriously affected because the whole fish including viscera is generally ingested (Rudd, 1964).

In 1964, several mink farms in southern Ontario suffered losses through failures in reproduction. Unconfirmed reports linked the failures with the presence of pesticide residues in the mink diets (C. R. Browne, 1965, private communication). Freshwater fish was considered as a possible source of the pesticide, and this lead to a sharp decline in the marketing of freshwater fish for mink food. In 1965, an investigation was carried out by the Fisheries Research Board of Canada on the nutritive value and composition of fish meals and oils from freshwater fish (Ackman et al., 1967; March et al., 1967); this provided an opportunity for determining the pesticide residue content of the following species: alewife (*Alosa pseudoharengus*) from Lake Michigan; sheepshead (*Aplodinotus grunniens*) from Lake Erie; tullibee (*Coregonus artedii*) from Lake Winnipeg; and maria (*Lota lota*) from Lake Winnipeg. The concentrations of heptachlor, heptachlor epoxide, DDE, DDT, DDD, and dieldrin in the meals and oils resulting from the rendering operations were determined[1] by gas and thin-layer chromatography. The meals and the oils were also screened for lindane, endrine, and methoxychlor but none of these residues was found. The results of analysis showed that DDT and its metabolites accounted for almost all of the pesticide residues in meals and oils. The actual results are listed in Tables I and II. From values shown in Tables I and II, the residue content of the bulk of the meals and of the oils was calculated; relating these figures to the initial weight of raw material used,

[1]The pesticide residue determinations were carried out under contract by the Ontario Research Foundation, Toronto, Ont.

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TABLE I. Analyses of fish meal samples for residues of common chlorinated hydrocarbons.

Sample	Pesticide residues in ppm[a]					
	Heptachlor	Heptachlor epoxide	DDE	Dieldrin	DDD	*p,p'*-DDT
Sheepshead	ND[b]	Trace[c]	0.020	Trace	0.065	Trace
Maria	ND	ND	0.044	ND	0.030	0.020
Tullibee	Trace	0.022	0.019	Trace	0.023	0.014
Alewife[d]	ND	0.011	0.519	Trace	0.146	0.361

[a]These samples were screened for lindane, endrine, and methoxychlor, and none was found.
[b]ND = <0.001 ppm.
[c]Trace = <0.01 ppm.
[d]This sample contains a small amount of *o,p'*-DDT

TABLE II. Analyses of oil samples for residues of common chlorinated hydrocarbons.

Sample	Pesticide residues (*ppm*)[a]			
	DDE	Dieldrin[b]	DDD	*p,p'*-DDT
Sheepshead	0.889	0.080	2.08	0.930
Maria	1.12	0.035	0.725	0.800
Tullibee	0.400	0.075	0.160	0.351
Alewife	11.2	0.066	12.5	0.392

[a]The samples were screened for heptachlor, heptachlor epoxide, lindane, endrine, and methoxychlor, and none was found.
[b]Dieldrin values were not confirmed on thin-layer chromatography.

the pesticide content of whole fish was calculated. The figures so obtained are presented in Table III and compared with those of corresponding meals and oils. The figures for whole fish are only approximate because some pesticide losses may have occurred in the course of the pressing out operation during meal manufacture. Table III shows that the pesticide residue is mostly in the oil, which is normal, since pesticide residues are generally located in the visceral fat in fish (Mawdesley-Thomas and Leahy, 1967; Rudd, 1964), and the oily portion of the fish is in greater part separated from the meals during rendering. Thus it is not surprising that the residue levels in the meals were about the same as those in the whole fish despite the fact that rendering causes a five-fold increase in solid matter.

TABLE III. Total chlorinated hydrocarbon residues in ppm.

	Fishmeal	Oil	Whole fish (calculated)
Sheepshead	0.085	3.979	0.154
Maria	0.094	2.680	0.014
Tullibee	0.078	0.986	0.044
Alewife	1.037	24.158	1.600

The calculated pesticide content in whole maria (*Lota lota*) from Lake Winnipeg (0.014 ppm) is in the range of values reported by J. J. Hickey (1967, private communication) for different species of fish from various lakes in the Province of Manitoba; the calculated content in whole alewife (*Alosa pseudoharengus*) from Lake Michigan is only slightly lower (1.6 ppm) than the value reported by Hickey et al. (1966) for the same species from that same lake (3.3 ppm).

The levels of chlorinated hydrocarbon pesticide residues in the meal and oil samples were, with the exception of the oil sample from Lake Michigan alewife, below the regulatory tolerances established by the Food and Drug Directorate of Canada (1965) for certain foods (including many crops and the fat of cattle, hogs, and sheep) intended for human consumption. It is unlikely, therefore, that the pesticide residues in raw fish or in meals would present any problem in relation to the use of fish and fish products in animal feed.

The samples which were used for these determinations were drawn from bulk samples of meals and oils which were obtained under commercial conditions by rendering 4 tons of whole fish for each species. Thus, the pesticide levels found in this investigation could be interpreted as being representative for each species. However, despite their relatively large size, the fish samples should be considered as representing local conditions, since distribution of pesticide residues in lake waters, and concentration by fish populations, are likely to vary from area to area in a large lake, according to the types of lands and their use in the different drainage systems, and in relation to local climatic conditions.

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Hematological Correlations in the Rainbow Trout, *Salmo gairdneri*

Hematological data have found increasing use among fisheries biologists in recent years as a basis for the assessment of dietary adequacy (Kamra, 1966; Poston, 1954; Schumacher, 1958) and the incidence of pathological states (Phillips et al., 1958; Telitchenko and Govorova, 1962). Information of this type also appears to offer some potential for the evaluation of physiological responses to certain forms of stress (Anthony, 1961; Bouck and Ball, 1966; DeWilde and Houston, 1967; Prosser et al., 1957; Snieszko, 1958). As a consequence, some considerable effort has been directed toward the development of analytical and enumeration procedures suitable for use with fish blood (Anthony, 1961; Hesser, 1960; Larsen, 1964; Larsen and Snieszko, 1961) and the establishment of hematological norms for various species (Eisler, 1965; McKnight, 1965; Schiffman and Fromm, 1959; Summerfelt et al., 1967).

Of the commonly employed hematological parameters, three (erythrocyte numbers, packed cell volume, hemoglobin concentration) can be estimated directly. These values may then be used to calculate a variety of other useful indices including the mean erythrocytic volume and hemoglobin content (Wintrobe, 1956). Because red cell enumeration and hemoglobin determination are not readily carried out in the field or with the facilities commonly available in hatchery installations, attempts have been made to establish predictive correlations between these parameters and the more readily determined hematocrit or microhematocrit (Eisler, 1965; Summerfelt et al., 1967).

In a recent study of thermoadaptive responses in rainbow trout (DeWilde and Houston, 1967) values for each of the three directly determined parameters were obtained for a group of 136 yearling fish. The sampling program included animals of both sexes, covered a weight range of 18.9–180.7 g, and involved summer and winter fish acclimated to temperatures varying from 3 to 21 C. Although the feed type was constant it may be assumed that differences in nutritional status corresponding to the different temperature regimes used also existed. Consequently, the data, taken as a whole, probably provide a reasonably close approximation to the conditions likely to be encountered in a natural population. Accordingly, correlation and regression analyses were conducted to provide additional information regarding the relationships between microhematocrit and erythrocyte and hemoglobin levels.

The animals used in the study were taken from a single yearling stock of trout hatched and reared at the Nevin Station of the Wisconsin State Conservation Department. Details pertinent to their maintenance and to sampling and analytical procedures have been outlined in an earlier report (DeWilde and Houston, 1967). Frequency distribution studies and correlation and regression analyses were carried out at the University Computing Center in accordance with programs devised by Monroe and Walsh (1967).

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Population means for red cell number ($1.293 \pm 0.016 \times 10^6$ cells/mm^3), microhematocrit (31.58 ± 0.27%), and hemoglobin concentration (7.42 ± 0.15 g/100 ml of whole blood) were reasonably close to those obtained in earlier studies upon this species (Bouck and Ball, 1966; Shiffman and Fromm, 1959). Correlation coefficients significant at $P > 0.01$ were found between erythrocyte concentration and packed cell volume ($r = 0.656$), erythrocyte concentration versus hemoglobin content ($r = 0.676$), and microhematocrit and hemoglobin concentration ($r = 0.765$). Correlation coefficients calculated for weight versus red cell count ($r = 0.079$), microhematocrit ($r = 0.066$), and hemoglobin ($r = 0.106$) were not significant.

Relationships between the various parameters are summarized in Table I. These are largely self-evident and require little comment. It may be noted that inclusion of specimen weight in the analysis provided little improvement

TABLE I. Results of multivariate analysis.

Parameter relationships	(SE/$\bar{M}$) × 100[b]	Variance: Parameter 1	Parameter 2	Parameter 3	Total
HB (±0.72)[a] = −1.349 + 0.278 PCV	9.7%	58.5%	–	–	58.5%
(±0.70) = −1.755 + 0.281 PCV + 0.004 WT	9.4%	58.5%	2.5%	–	61.0%
(±0.82) = 1.993 + 4.196 RBC	11.1%	45.%	–	–	45.4%
(±0.82) = 1.931 + 4.170 RBC + 0.002 WT	11.1%	45.4%	0.3%	–	45.7%
(±0.67) = −1.508 + 0.206 PCV + 1.879 RBC	9.0%	58.5%	5.2%	–	63.7%
(±0.66) = −1.812 + 0.215 PCV + 1.714 RBC + 0.004 WT	8.9%	58.5%	5.2%	1.4%	65.1%
RBC (±0.135) = 0.084 + 0.038 PCV	10.4%	43.1%	–	–	43.1%
(±0.133) = 0.033 + 0.038 PCV + 0.001 WT	10.3%	43.1%	1.5%	–	44.6%
(±0.132) = 0.490 + 0.108 HB	10.2%	45.4%	–	–	45.4%
(±0.132) = 0.488 + 0.108 HB + 0.0004 WT	10.2%	45.4%	0%	–	45.4%
(±0.127) = 0.174 + 0.066 HB + 0.020 PCV	9.8%	45.4%	4.8%	–	50.2%
(±0.126) = 0.144 + 0.063 HB + 0.021 PCV + 0.0002 WT	9.7%	45.4%	4.8%	0.3%	50.6%

[a]Figures in parentheses represent ± standard error. RBC = erythrocyte numbers, 10^6 cells/mm^3; PCV = packed cell volume, %; HB = hemoglobin, g/100 ml blood.
[b]Standard error as a percentage of the mean.

in any of the relationships derived. Indeed, the weight coefficients are not significant, and are included only for illustrative purposes. Although the standard errors of estimate for erythrocyte levels (±9.7 to 10.4% of population mean) are surprisingly close to the methodological error of the enumeration procedure (Wintrobe, 1956), only 43.1% of the total variance of the data was

accounted for in the simplest relationship considered (erythrocyte number vs. hematocrit). Inclusion of additional parameters provided no substantial improvement. Thus, predictions of red cell numbers from hematocrit, hemoglobin, and weight still account for only 50.6% of the variance.

Errors of estimate for predictions of hemoglobin concentrations ranged from ±8.9 to ±11.1% of the population mean. These are, as might be anticipated, considerably in excess of both normal methodological error and population variability (DeWilde and Houston, 1967). Erythrocyte counts clearly provide the poorest means of estimating hemoglobin content in terms of both standard error (11.1%) and variance (45.4%). More reliable predictions are obtained through the use of microhematocrits (standard error = ±9.7%, variance = 58.5%). Employment of three variables (microhematocrit, erythrocyte number, weight) resulted in a reduction of the standard error to 8.9%, and increased the variance accounted for from 58.5 to 65.1% of the total. The improvement is, however, not sufficient to justify the increase in time expenditure needed for red cell enumeration.

In view of the correlations, standard errors, and variances observed, it seems reasonable to conclude that, at least for the rainbow trout, microhematocrit values may be substituted for erythrocyte counts and hemoglobin determinations in routine assessments of hematological status. The reliability of hemoglobin and red cell predictions is, however, not sufficiently exact for research purposes.

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A Harpoon Adapter for Tagging Large Free-Swimming Fish at the Surface

The tagging of large pelagic fish presents problems due to their size and the method of capture. This is particularly true for swordfish and large bluefin tuna, both of which are mainly taken on longlines. Fish that are left on the lines rapidly become exhausted and the majority (95–100% for swordfish) are dead, or apparently moribund, on hauling. It is therefore desirable to mark these fish without first capturing them, and an obvious method is by means of a harpoon when they are basking at or near the surface. Since 1962 members of the Fisheries Research Board of Canada's Biological Station at St. Andrews, N.B., have tried tagging with a harpoon on several cruises of research and commercial vessels, but with little, if any, success. These early attempts used a standard commercial harpoon modified to carry a tag applicator and a "stopper," to prevent over-penetration. When thrown, however, the harpoon shaft fell over after hitting the fish and the applicator was torn out of the flesh. This problem could be avoided by retaining the harpoon in hand and using a quick stabbing motion, but this severely limited the effective range.

An adapter (Fig. 1) has now been developed that fits onto the iron harpoon shaft in the place of the dart, and allows the harpoon to be thrown without jeopardizing tag retention. The adapter releases the tag and applicator immediately after they have been inserted into the fish. The applicator is thus

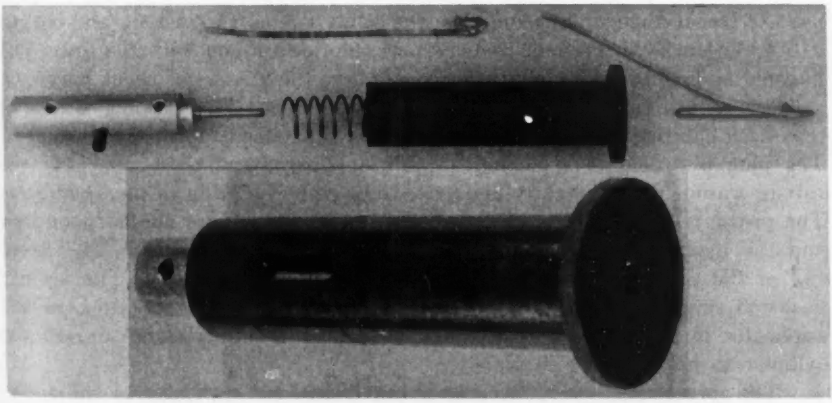

FIG. 1. Harpoon adapter for tagging large pelagic fish. *Top*, partially exploded view showing the piston complete with set screws, guide bar, and push-rod, and the spring, cylinder and applicator, and tag. A second tag is shown to indicate the type of dart in use. *Bottom*, oblique view of assembled adapter.

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left in the flesh, but as it is a solid stainless steel rod for the tags currently in use (Woods Hole type metal anchors with various message parts), without any barbs or other retaining devices, it will presumably work out and leave the tag behind.

The general design of the adapter is shown in the accompanying diagram (Fig. 2). Basically the attachment for the harpoon is a piston which travels

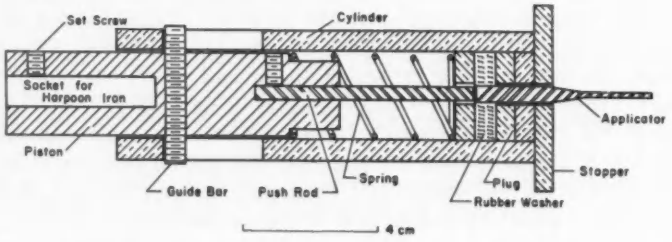

FIG. 2. Diagrammatic section of harpoon adapter to scale.

within a cylinder holding the tag applicator. The momentum of the harpoon via the piston and a push-rod drives the applicator out of its mounting against the resistance of the flesh. The piston is retained in the cylinder by a guide bar running in slots cut in the cylinder wall and is held in the undischarged position by a spring. The spring also prevents premature discharge due to the resistance of the water. The exposed length of the push-rod at the lower end of the piston can be adjusted so that it is level with the outer edge of the stopper when the applicator is expelled.

As constructed at St. Andrews, the applicator is 16 cm long and weighs 210 g. The cylinder is made of polyvinyl chloride tubing, with the lower end plugged and capped. The plug consists of three thicknesses of sheet polyvinyl chloride with a rubber washer between two of them. The capping is also of polyvinyl chloride sheet and can be of larger diameter to form the "stopper." The plug is drilled to accommodate the applicator and push-rod, with the rubber washer drilled slightly undersize to provide retention of the applicator. The piston is of aluminum alloy drilled at opposite ends for the harpoon iron and the push-rod. Both recesses are furnished with set screws. The lower end of the piston is turned down to accommodate the spring. The spring, push-rod, and guide bar are of stainless steel. Additional retention of the applicator in its mounting is provided by use of a rubber band around the cylinder to hold the tag in place.

The spring must be sufficiently heavy to prevent expulsion of the applicator by the resistance of the water on the front of the adapter. Also it is important that the travel of the piston be slightly less than the thickness of the plug and stopper so that the push-rod will remain in the hole through the plug when the adapter is loaded.

The adapter has been used on 21 occasions resulting in the tagging of two free-swimming bluefin tuna and of 19 longlined fish (16 blue sharks, 1 mako shark, 1 dusky shark, and 1 dolphin). There have been no recoveries to date, but the tagging and subsequent examination of dead fish has suggested that tags are set satisfactorily. There has, therefore, been little assessment of the success of the method, but it is felt that the principle will be of interest to other agencies involved in tagging free-swimming fish.

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Two New Specimens of the Notacanthid Fish *Macdonaldia challengeri* in the Eastern North Pacific Ocean

During investigations by the Fisheries Research Board of Canada's research vessel *G.B. Reed* off northern Vancouver Island, two specimens of the notacanthid fish *Macdonaldia challengeri* (Vaillant, 1888) were taken. They were captured in a "Gulf Shrimp Trawl" at depths between 1150 and 1200 fath (2103–2196 m) west of Triangle Island at lat 50°54.5′N, long 130°06′W on September 11, 1964, and are now deposited in the museum of the Institute of Fisheries, The University of British Columbia (collection number: BC64-444).

The species was originally described from a specimen taken off Japan (lat 34°N, long 140°E) at a depth of 3429 m. A second specimen (originally recorded by Gilbert, 1896, and Jordan and Evermann, 1896) is listed by Grey (1956) from the Bering Sea (lat 56°N, long 172°W) in depths of 2971 m. The additional two specimens recorded here from waters off British Columbia indicate that the species is probably widely distributed in the more northern parts of the Pacific Ocean. The apparent rarity of known specimens is undoubtedly due to infrequent explorations of the great depths in which they live.

Two other notacanthid fishes — *M. longa* and *M. alta* (*M. alta* = *Polyacanthonotus altus*: Trotti, 1943; Grey, 1956) — have also been described from the Bering Sea by Gill and Townsend (1897) and Jordan and Evermann (1898). The holotypes of these forms as well as that of *P. vaillanti*, an Atlantic form described by Fowler (1934), were examined by the author at the United States National Museum. Table I presents the counts and measurements of these specimens along with data taken from the literature on other specimens which also belong to the notacanthid subfamily Polyacanthonotinae. For easier comparison, data taken from the literature have been transformed to the style presented in Table I. Differences in techniques of measurement make many of these figures only roughly comparable with those of the specimens examined in this report. For example Trotti (1943) uses total length for comparison of measurements rather than the standard length used by other workers. Trotti (1943) also records different measurements for several characters (i.e. eye diameter and snout length) compared with those presented in this note. This suggests that a proper evaluation of the Polyacanthonotinae can only be made when all the known specimens become available for reexamination of these characters.

Unless stated otherwise, counts and measurements for specimens examined by the author follow the definitions of Hubbs and Lagler (1958). The distinction between spinous and soft anal fin rays in specimens from collection BC64-444 was determined from radiographs. Spinous rays are considered

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TABLE I. Comparison of counts and measurements in *Macdonaldia* and *Polyacanthonotus*. Bold face figures are those measured by the author. Measurements expressed as hundredths.

	P. altus USNM 48774 holotype	*P. rissoanus* Trotti (1943)	*P. vaillanti* USNM 44248 holotype	*P. vaillanti* Trotti (1943)	*M. challengeri* BC64-444 #1	*M. challengeri* BC64-444 #2	*M. challengeri* Jordan and Evermann (1896)	*M. challengeri* Trotti (1943)	*M. longa* USNM 48775 holotype	*M. africana* Trotti (1943)	*M. rostrata* Trotti (1943)	*Macdonaldia* sp. Trotti (1943)
No. of specimens	**1**	22	**1**	–	**1**	**1**	1	–	**1**	1	–	1
Dorsal fin spines	**32**	26–34	**37**	–	**33**	**32**	35	34	**33**	36	27–35	36
Spinous anal fin rays	52[a]	32–45	–	37 or 56	**59**[d]	**39**[d]	40	54	55[a]	47	32–53	44
Spinous anal rays under dorsal fin	**32**	–	**27**	–	**35**	**26**	–	–	**26**	–	–	–
Soft anal fin rays	125[a]	?	–	120?	**126**	**136**	140	79	111[a]	–	192–205	121
Precaudal vertebrae	–	–	–	–	**57**	**55**	–	–	–	–	–	–
Caudal vertebrae	–	–	–	–	–	**187** approx.	–	–	–	–	–	–
Vertebrae anterior to last dorsal spine	–	–	–	–	**104**	**103**	–	–	–	–	–	–
Pectoral rays	**14**	12–16	**14**	13	**13**	**12**	11	11	**13**,(10[b])	12	14–15	14
Pelvic rays	I+9	I+6–I+10	**I+9**	–	**I+8**	**I+8**	I+9	I+9	**I+9**	I+9	10–I+10	II+8
Caudal rays	–	3–6	–	–	**5**	**5**	5	6[a]	–	–	–	–
Standard length (*mm*)	**460**	158–230	**488**	260–500	**372**	**467**	396[c]	508[c]	**526**,(532[b])	–	180–408	321
Preanal distance in standard length	**38.6**	$\bar{x}$=33.3	**39.1**	41.3	**39.7**	**38.9**	–	41.3	**40.2**	–	33.3–37.0	40.0
Predorsal distance in standard length	**14.6**	–	**18.2**	–	**14.9**	**15.9**	–	–	**14.6**	–	–	–
Body depth at origin of anal fin in standard length	**10.4**	$\bar{x}$=7.3	**10.4**	7.7–10.7	**8.2**	**7.1**	–	8.0	**7.9**(8.3[b])	–	6.1–7.1	8.7
Body depth at pelvic fin base in standard length	**9.6**	–	**10.0**	–	**7.7**	**7.5**	–	–	**7.6**	–	–	–
Head length in standard length	**12.4**	$\bar{x}$=13.8	**11.2**	12.5	**13.3**	**13.6**	–	13.2	**12.1**	14.3	12.8–11.8	14.3
Body depth at pectoral fin base in standard length	**6.6**	–	**6.0**	–	**6.1**	**6.1**	–	–	**6.1**	–	–	–
Body depth at pos-												

Body depth at pectoral fin base in standard length	6.6	–	6.0	–	6.1	6.1	–	–	6.1	–	–	–
Body depth at posterior part of orbit in standard length	3.5	–	2.8	–	3.8	3.6	–	–	3.7	–	–	–
Body width in standard length	5.0	–	4.9	–	4.3	4.4	–	–	3.4	–	–	–
Body depth at origin of anal fin in preanal distance	26.9	–	26.6	–	20.6	18.2	–	–	19.6	–	–	–
Pelvic base to anal base in preanal distance	12.7	–	17.0	–	11.1	14.5	–	–	13.4	–	–	–
Eye diameter in head length	13.5	$\bar{x}$=14.7	12.9	18.3	11.7	11.3	–	16.1	14.1(15.6[b])	13.3	15.0-15.2	13.3
Eye to end of opercle in head length	60.4	–	58.8	–	60.7	59.3	–	–	59.6	–	–	–
Snout length in head length	28.8	$\bar{x}$=38.5	28.2	32.0	30.5	31.0	–	32.3	27.6(31.2[b])	33.3	33.3–37.5	33.3
Anterior border of mouth to tip of snout in head length	14.2	$\bar{x}$=16.0	13.7	–	15.4	18.8	–	–	13.8(20.0[b])	–	11.2–13.0	16.6
Interorbital width in head length	9.4	–	7.2	–	9.3	9.9	–	–	7.7	–	–	–
Length of upper jaw in head length	18.4	–	14.0	–	17.8	17.0	–	–	18.5	–	–	–
Pectoral fin length in head length	37.1	–	36.4	–	34.0	40.3	–	–	32.6	–	–	–
Pelvic fin length in head length	30.6	–	34.1	–	29.8	37.5	–	–	30.9	–	–	–
Pectoral base to lateral line in distance from pectoral base to operculum	104.4	–	54.8	–	47.3	63.3	–	–	54.1	–	–	–

[a]Jordan and Evermann (1896, 1898).
[b]Trotti (1943).
[c]Grey (1956).
[d]Anal counts are LVII, 128 for 372-mm specimen and LXVII, 107 for 467-mm specimen when technique of Marshall (1962) is employed.

to be those without any articulations across the ray. Anal fin counts for specimens other than BC64-444 are those recorded previously in the literature. Caudal vertebrae become very reduced posteriorly and could not be counted with certainty.

More recent authors (i.e. Trotti, 1943; Grey, 1956) have recognized the following forms of the Polyacanthonotinae: *P. altus*, *P. rissoanus*, *P. vaillanti*, *M. challengeri*, *M. longa*, *M. africana*, and *M. rostrata*. *Polyacanthonotus vaillanti* probably has appreciably more dorsal fin spines and *P. altus* might be deeper and possess a differently placed pectoral fin compared with the other forms of *Macdonaldia* and *Polyacanthonotus* listed in Table I. However, for the most part there is wide variation in many of the characters in the specimens of each species so that without larger samples it is impossible to determine the number of species involved. There is very great variation in the fin ray counts of the 22 specimens of *P. rissoanus* reported by Trotti (1943). Also both specimens in BC64-444 differ widely, especially in anal fin counts (LIX, 126, to XXXIX, 136). If the disappearance of articulations between lepidotrichia is used as a definition between spinous and soft anal rays then there is a difference of at least 20 spinous rays. If the definition of Marshall (1962) is employed in which spinous and soft rays are determined according to which has a pointed tip, then there is still variation (LVII and LXVIII). Previous authors (i.e. Jordan and Evermann, 1896, 1898) have used spinous anal fin rays as a diagnostic character. The described species and specimens of the Polyacanthonotinae are known from localities that are far apart and consequently geographic variation within species is possible. Since so few specimens are known for most of the described forms, few conclusions can be made on the variability and species limits within the Polyacanthonotinae. It is preferable to identify specimens BC64-444 with the earlier described form from the Pacific Ocean as *M. challengeri*.

Description of *Macdonaldia challengeri* from British Columbia

Body: very elongate; slender; greatest depth at origin of anal fin. Head: slightly compressed; dorsal profile slightly convex over eye, otherwise straight. Eye: small; well below dorsal profile of head; anterior border directly over posterior edge of maxillae; covered by thick skin which is probably transparent in life. Snout: rostrum-like; dorsal profile straight, ventral profile rounded to inferior mouth. Mouth: small; lower jaw shorter than upper jaw; maxillae, each with a strong posteriorly directed spine on posterodorsal edge. About 30–35 small, sharply pointed teeth of relatively uniform size arranged mostly in a single row on each premaxillary, dentary, and palatine bone (Fig. 2). These teeth arranged across full width of mouth. Mandibular teeth fit between rows of premaxillary and palatine teeth. Tongue with a few scattered, small papillae on upper surface. Roof of mouth in larger preserved specimen quite fleshy. Fleshy longitudinal swellings or ridges lead into deeper fleshy groove in midline of roof of mouth in this specimen. This groove deepens

FIG. 1. Sketch of 467-mm specimen of *Macdonaldia challengeri* (BC64-444).

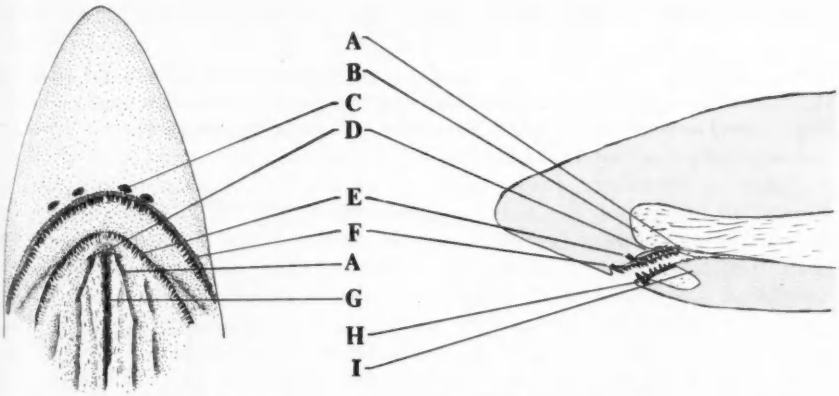

FIG. 2. Oral morphology of 467-mm specimen of *Macdonaldia challengeri*: *left*, diagrammatic view of roof of mouth from ventral view; *right*, mid-sagittal view. A = longitudinal ridge at entrance to groove, B = pouch, C = premaxillary head pore, D = secondary oral valve-like structure, E = palatine tooth series, F = premaxillary tooth series, G = groove, H = mandibular teeth, I = tongue.

as it leads anteriorly toward a blind pouch, the latter situated directly above palatine tooth series. A longitudinal soft fleshy ridge appears to line entrance to groove and pouch. Anteriorly, some loose skin (almost like a reduced secondary oral valve) stretches across between these ridges. The valve-like structure is not so apparent in smaller specimen. A few very small papillae are arranged in a row on each ridge directly behind this oral valve-like structure. In smaller specimen the papillae are much more developed and as well as being more numerous are several times longer than broad. Many of these papillae in small specimen are club-shaped and lie in rows anterior to entrance of pouch. A few smaller papillae arranged linearly and longitudinally along fleshy ridges in roof of mouth (Fig. 3). A fleshy ridge is present in typical position of the oral

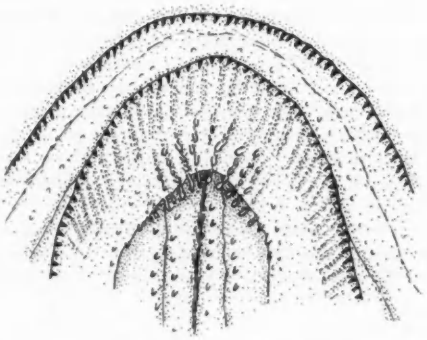

FIG. 3. Diagrammatic view of the roof of the mouth in the 372-mm specimen of *Macdonaldia challengeri* (BC64-444). Note greater size and abundance of papillae.

valve of other teleost, but this ridge is not as membranous as a typical oral valve would normally be. Operculum; large, extending far posteriorly. Gill rakers: 4 + 10; elongate and pointed on first two gill arches, short and stubby on last gill arch.

Nostrils: large, anterior to eye and over posterior half of mouth; anterior nostril tubular and short; posterior nostril with anterodorsal flap which makes the opening face posteroventraly (Fig. 4). Head pores: fairly conspicuous; pores above and below mouth very large and prominent;

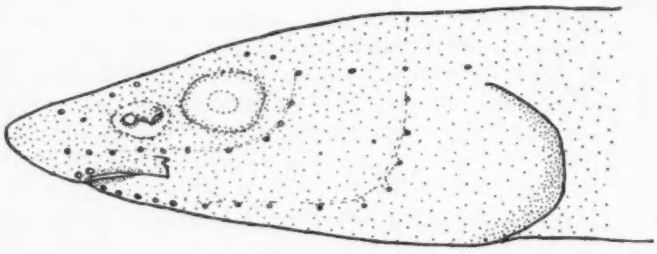

FIG. 4. Diagram showing relative position of head pores and nasal aperture (a few head pores are not shown).

15 pores in mandibular to preopercular series (on smaller specimen); 4 in postorbital series; 2 on premaxillary series; 12 on suborbital to snout series; 6 or 7 on superorbital to snout series. Lateral line: prominent; high over anterior portion of body but bisects the lateral side into equal halves over the anal fin; terminates at a distance equal to one and a half times the head length before the caudal fin. Lateral line pores difficult to count but approximately 140.

Scales: imbricated, small, cycloid; covering all of body except posterior margin of gill cover, branchiostegal membrane, posterior portion of maxillary bones, and lips of both jaws; also absent on pectoral, pelvic, caudal, and dorsal fins, and posterior soft-rayed portion of anal fin.

Dorsal fin: composed of widely spaced, short, stout, sharp spines; no membrane beween spines; sharp splint attached to but hidden by last spine (splint not included in dorsal count). Anal fin: long, originates immediately behind anus; anteriorly, spines short, strong, sharp, widely spaced; posteriorly, spines grade into moderately long, flexible, soft rays; anteriorly, margin of fin membrane covers base of exposed spines only but posteriorly the margin of the membrane unites tips of spines and soft rays. Pectoral fins: laterally placed; rays on dorsal side longer than those on ventral side of fin; fleshy base of pectoral, elongate, slightly elevated; pectoral rays branched. Pelvic fins: abdominal; moderately short; membrane not confluent between each pelvic fin; soft rays branched; spinous ray very short and sharp; distance of pelvic insertion to center of anus equal to length of pelvic fin. Caudal fin; very small, base narrow, rays simple, membrane confluent with that of anal fin.

Colour: light; after several weeks in formalin cream to faintly pink; some areas appear discoloured with light brown; posterior portion of anal and caudal fin black. Lips, posterior edges of gill cover, lining of mouth, gill chamber, peritoneal lining of oesophagus and cardiac portion of stomach, black.

Both specimens reported here permit few conclusions on the biology of *M. challengeri* other than that they are deepwater benthic forms. The larger specimen appeared to be a female and contained a few remnants of arthropods in the stomach.

The prominent lateral line, head pores, nostrils and oral papillae suggest well-developed acoustic, gustatory, and olfactory senses. The form of the roof of the mouth and its anteriorly placed pouch is most peculiar. Their function is completely unknown. A storage space for food might be postulated but there is no apparent reason for the need of such a structure. Gross morphology does not reveal a mechanism in the preserved specimens that moves material into or out of this pouch. The tongue does not extend quite as far as the entrance of the pouch. It is possible that the pouch was inevitably left as a by-product after the palatine bones shifted ventrally in relation to the other bones of the skull. However, one is forced to the conclusion that the space which was consequently left for this pouch must have a significant effect on the functioning of the mouth.

Living specimens must be observed to determine whether the ridges and secondary oral valve-like structure can function similar to the typical oral valve of other teleosts or whether these structures regulate materials into or out of the pouch. The combination of the secondary oral valve and fleshy longitudinal ridges could conceivably provide a seal against the lower jaw and tongue in order to prevent reverse flow of water during respiration. Most teleosts have an oral valve between the premaxillary and palatine teeth and not posterior to the palatines. In the specimens reported here the skin between the palatine and premaxillary teeth is loose and might contribute to form a seal; however, no typical oral valve is present. There is no ventral oral valve on the lower jaw but this is a common feature of teleosts which are similar to *M. challengeri* in having inferior mouths. The extreme inferior nature of the mouth and shortened lower jaw might not permit an effective seal by an upper valve between the premaxillae and palatines. Since the teeth of the lower jaw fit between the upper two sets of teeth when the mouth is closed, an upper oral valve in its usual position might possibly be torn. Thus a seal posterior to the palatines might be more adaptive. In light of the very well-developed papillae of the smallest specimen in BC64-444 the main function of the ridges near the pouch could involve the chemoreception of food.

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DDT Residues and Metabolites in Canadian Atlantic Coast Fish

The following study was undertaken to determine the concentrations of DDT residues and metabolites in fish caught near the Atlantic coast of Canada: in mackerel caught in the Gulf of St. Lawrence, off Prince Edward Island; in young salmon found dead in streams of the Miramichi region of New Brunswick which had been aerially sprayed with DDT to control the spruce budworm; and in brook trout from freshwater streams in Prince Edward Island.

Material and methods.— The concentrations of the following were measured in fish: *p,p'*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane) and *o,p'*-DDT (1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)-ethane) along with two metabolites of *p,p'*-DDT namely *p,p'*-DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene) and *p,p'*-DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane). The compound *o,p'*-DDT is an isomer of *p,p'*-DDT and may constitute from 8 to 21% of technical DDT; a small amount of DDD is also present in technical DDT (Haller et al., 1945).

The brook trout, *Salvelinus fontinalis*, were caught in Prince Edward Island freshwater streams. They were 15 cm in length and weighed approximately 75 g. The streams in which the trout had been caught were not sprayed with DDT but possibly had drained agricultural land sprayed with this insecticide. Trout tissue (10 g) were taken for analysis.

Mackerel, *Scomber scombrus*, 30 cm long and weighing approximately 600 g were analyzed. The mackerel viscera analyzed consisted of a sample of a homogenate of the internal organs of the fish. Generally 10 g of mackerel tissue were taken for analysis. The livers weighed 2–6 g and the brains 0.7–1 g.

Atlantic salmon, *Salmo salar*, 15 cm long and weighing approximately 20 g were investigated. Salmon No. 14 to 16 (Table III) were obtained from rivers in an area where DDT had not been applied. Ten grams of tissue were used for analysis.

All fresh fish samples were stored at –20 C.

Gas chromatography was used for the analyses, the analytical instrument being a Wilkens Aerograph Hi-Fi Model 600-C equipped with an electron capture detector. The analytical column used and operating conditions employed are described by McCully and McKinley (1964).

The weighed samples were placed in a Waring blendor and extracted according to the procedure of Onley (1964). The petroleum ether layer containing the residues and metabolites was concentrated to 5 ml.

The concentrated sample was chromatographed on a 10-cm Florisil column. The concentrate was transferred to the column and the insecticides eluted with 75 ml of elution mixture 1 (120 ml of ethyl ether containing 2% ethyl alcohol diluted to 1000 ml with petroleum ether) followed by 125 ml of

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elution mixture 2 (50 ml of ethyl ether containing 2% ethyl alcohol, plus 4 ml of dioxane, diluted to 1000 ml with petroleum ether). The combined volumes were flash evaporated and diluted to 5 ml, and an aliquot directly injected into the gas chromatograph.

The analytical method employed would detect *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE at concentrations at or above 0.01 ppm based on total wet weight.

Recovery experiments were carried out by adding *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE to fish homogenate, extracting the homogenate, and purifying the extract using column chromatography.

The average recovery and the recovery range for three determinations for each of the added chemicals were:

Insecticide	Average recovery, %	Recovery range, %
p,p'-DDT	90	85–105
o,p'-DDT	81	75–95
p,p'-DDD	82	75–95
p,p'-DDE	87	80–100

A sample of the same homogenate was used as a blank sample and any background peaks in this sample due to presence of insecticides were subtracted from the spiked homogenate to obtain the final recovery results.

Salmon flesh was analyzed also by thin-layer chromatography (Walker and Beroza, 1963), for comparison with the findings by gas chromatography. An aliquot of the purified eluate equivalent to 5 g of fish sample was evaporated and spotted on a chromatographic plate. Known amounts of the pure insecticides together with spiked samples of fish extract were spotted and chromatographed simultaneously with the unknown samples. The chromatographic plate was placed in a tank and developed with hexane. The tank and contents were kept at 10 C. The plate was removed from the tank, air dried, sprayed with silver nitrate, and exposed to ultraviolet light for 5 min. The amount of insecticide in the unknown sample was visually estimated by comparing spot size and intensity with those from known quantities of insecticides chromatographed on the same plate. The R_f values of the insecticides were as follows: *p,p'*-DDT, 0.24; *o,p'*-DDT, 0.28; *p,p'*-DDD, 0.17; *p,p'*-DDE, 0.37.

Results.— The chief chemical found in trout flesh was *p,p'*-DDE (Table I). The concentrations were higher in the viscera than in the flesh. Three of the four viscera samples analyzed contained*o, p'*-DDT at concentrations from 0.03 to 0.16 ppm. A single sample of viscera contained *p,p'*-DDD, *o,p'*-DDT, and *p,p'*-DDT.

In mackerel (Table II) higher concentrations of *p,p'*-DDT were found in the flesh and liver (<0.01–2.75 ppm) than in the roe or brain (<0.01–1.98

TABLE I. Numbers of freshwater brook trout in which various concentrations of DDT residues and metabolites were found in the flesh and viscera.[a]

Concentration (*ppm*)[b]	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDE
		Flesh[c]		
<0.01	8	8	8	3
0.01–0.10	0	1	1	5
0.11–1.00	1	0	0	1
		Viscera		
<0.01	3	1	3	0
0.01–0.10	0	2	0	1
0.11–1.00	1	1	1	3

[a]Nine trout in all were sampled, the viscera being analyzed in four.
[b]Based on total wet weight of sample.
[c]Lean muscle tissue.

TABLE II. Numbers of mackerel in which various concentrations of DDT residues and metabolites were found in various tissues.[a]

Concentration (*ppm*)	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDE
		Roe		
<0.01	2	6	7	1
0.01–0.10	0	2	0	1
0.11–1.00	7	1	3	7
1.01–2.00	1	1	0	1
		Brain		
<0.01	9	13	12	4
0.01–0.10	0	0	0	1
0.11–1.00	3	2	2	3
1.01–2.00	4	1	0	0
>2.00	0	0	1	0
		Flesh[b]		
<0.01	2	7	10	3
0.01–0.10	0	2	0	1
0.11–1.00	8	7	4	12
1.01–2.00	4	0	2	0
>2.00	2	0	0	0
		Liver		
<0.01	5	14	10	4
0.01–0.10	0	0	0	1
0.11–1.00	10	2	6	8
1.01–2.00	0	0	0	3
>2.00	1	0	0	0

[a]Sixteen mackerel in all were sampled, roe being taken from 10.
[b]Lean muscle tissue.

ppm). Flesh was frequently found to contain *o,p'*-DDT, at concentrations from 0.03 to 0.69 ppm, whereas it was occasionally found in roe, brain, and liver samples. The highest concentration of *o,p'*-DDT (1.65 ppm) was found in brain tissue. *p,p'*-DDD was detected in 33% of the samples analyzed, at concentrations from 0.08 to 4.96 ppm. Higher concentrations of *p,p'*-DDE, from less than 0.01 to 5.38 ppm, were found in the brain than in the liver, flesh, or roe.

The salmon samples that were taken from the sprayed area (Table III, 1–13) all contained *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE. The major chemical found using gas–liquid chromatography was *p,p'*-DDE, at concentrations from 7.6 to 20.8 ppm. Concentrations of *p,p'*-DDT ranged from 0.6 to 6.8 ppm and those of *o,p'*-DDT and *p,p'*-DDD from 0.3 to 1.8 ppm and 0.6 to 2.4 ppm respectively.

TABLE III. Comparison of insecticide levels in salmon flesh using thin-layer and gas–liquid chromatography (ppm).

Salmon no.	Gas chromatography				Thin-layer chromatography[a]			
	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDE	*p,p'*-DDT	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDE
1	0.6	0.3	0.7	10.5	0.1	0.1	0.2	10
2	1.4	0.7	1.3	16.9	0.8	1.0	2.0	16
3	0.o	0.3	1.3	15.0	0.7	1.0	2.0	15
4	2.8	0.8	0.9	14.4	1.3	1.2	0.2	15
5	2.5	0.7	2.4	13.4	1.0	0.8	2.0	12
6	2.0	0.8	0.9	18.3	1.0	1.1	0.2	20
7	0.6	1.1	0.6	20.2	0.5	1.0	0.2	20
8	2.1	1.5	1.1	13.8	1.0	1.5	1.0	20
9	1.2	1.4	1.1	20.8	1.0	1.0	0.2	18
10	3.5	1.2	1.3	11.2	2.5	1.0	0.2	10
11	5.0	1.8	2.3	12.8	5.0	1.5	1.0	13
12	6.8	1.5	2.2	7.6	2.3	1.0	1.0	8
13	3.3	1.4	1.6	15.3	3.1	1.1	0.2	15
14	0.05	<0.01	0.03	0.2	<0.1	<0.1	<0.1	0.3
15	0.05	<0.01	0.03	0.1	<0.1	<0.1	<0.1	0.4
16	<0.01	<0.01	<0.01	0.3	<0.1	<0.1	<0.1	0.4

[a]Lowest detectable limit 0.1 ppm.

From gas–liquid chromatography results, the concentrations of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE in the salmon samples from an area not sprayed with DDT (Table III, 14–16) were less than 0.01–0.05 ppm; less than 0.01 ppm; less than 0.01–0.03 ppm; and 0.1–0.3 ppm.

Discussion.— Concentrations of *p,p'*-DDT in liver and flesh of mackerel were in the same range (<0.01–2.75 ppm). Holden (1966) reports concentrations of *p,p'*-DDT from 0.01 to 0.07 in muscle and 0.01 to 0.02 ppm in liver of perch

from contaminated Loch Leven. Levels for *p,p'*-DDT in sea trout which spent a great deal of their time in polluted estuarine and coastal waters ranged from 0.01 to 0.05 ppm in muscle and 0.01 to 0.10 in liver. These values are consistently lower than the concentrations found in mackerel. This is possibly related to the fact that mackerel have a higher oil content than trout. Allison et al. (1963, 1964) found that brain tissue of cutthroat trout exposed experimentally to *p,p'*-DDT was consistently high in lipid and low in *p,p'*-DDT. We found low concentrations of *p,p'*-DDT in mackerel brain, 9 samples out of 16 having less than 0.01 ppm.

In this study, *p,p'*-DDD was found in 33% of the mackerel samples analyzed. Allison et al. (1963, 1964) found *p,p'*-DDD in fish exposed to *p,p'*-DDT in a warmwater pond. The same authors report that several types of microorganisms convert *p,p'*-DDT-C^{14} to *p,p'*-DDD. Recently, Miskus et al. (1965) reported that *p,p'*-DDT-C^{14} was converted to *p,p'*-DDD in lake water. Boyle et al. (1966) indicated that DDD from technical DDT was concentrated in fish viscera. These authors reported that 22% of the total insecticide in the viscera of fish exposed to DDT was DDD whereas the original technical DDT contained only 2% DDD.

p,p'-DDD, at low concentrations, was consistently found in the salmon samples studied. Reductive dechlorination of *p,p'*-DDT to *p,p'*-DDD has been demonstrated in rat liver (Klein et al., 1964), yeast (Kallman and Andrews, 1963), and in the bacteria *Proteus vulgaris* (Barker et al., 1966) and *Serratia marcescens* and *Escherichia coli* (Stenersen, 1965). *p,p'*-DDD in fish therefore could be a metabolic product of *p,p'*-DDT before or after death or it could be produced at an earlier stage in the food chain.

The chemical *p,p'*-DDE has been commonly found in fish (e.g., Allison et al., 1963; Holden, 1966; Boyle et al., 1966). We found higher quantities in mackerel brain than in the flesh or liver. The concentrations found in liver were about 10 times greater than the highest reported by Holden (1966) in the liver of sea trout which spent considerable time in polluted estuaries. In the same study *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE were reported in perch caught in contaminated Loch Leven. The presence of *p,p'*-DDE suggests that *p,p'*-DDT is in part metabolized to *p,p'*-DDE in fish (Boyle et al., 1966) or that the conversion occurs in an aquatic environment and the chemical concentrated by the fish.

The high *p,p'*-DDE values we found in young salmon indicate that *p,p'*-DDE is concentrated by the fish from the aquatic environment or that *p,p'*-DDT is converted in part to *p,p'*-DDE in the fish before or after death.

Jeffries and Walker (1966) indicated that *p,p'*-DDE is the chief metabolite of *p,p'*-DDT in avian liver, and assumed that DDD residues were of postmortem origin since this product was not found shortly after death but appeared after the sample had been stored.

Cole et al. (1967) analyzed brook trout after aerial application of DDT at 0.5 lb per acre. They found *p,p'*-DDT at 0.20–9.5 ppm, *o,p'*-DDT at

<0.002–0.12 ppm, *p,p'*-DDD at <0.002–6.1 ppm, and *p,p'*-DDE at 0.33–4.8 ppm in a composite sample of 10 fish.

Allsion et al. (1963) consistently found *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD in cutthroat trout exposed to various known concentrations of *p,p'*-DDT The analyses of tissue and organ pools from dead fish indicated highly variable distributions of organochlorine compounds. It was their opinion that the distribution of insecticides in the fish organs did not seem to be a function of the lipid content. Their studies revealed no clear pattern of relative distribution of residues in tissue pools.

The gas chromatographic results for salmon were supported by thin-layer chromatography. The thin-layer chromatography results are qualitative and only semi-quantitative but good agreement was obtained for the two methods.

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Microorganisms From Some Tropical Fish Diseases

One of the major problems in pisciculture is that diseases may affect the fish, bacterial diseases being probably more prevalent and of greater economic significance than any others. Bacterial diseases, as well as miscellaneous diseases of uncertain origin, in fisheries have been reviewed by Davis (1953), Van Duijn (1956), and Reichenbach-Klinke and Elkan (1965).

An attempt was made in the present studies to isolate microorganisms from diseased fish found in tropical waters and to test the isolates for pathogenicity.

Materials and methods.— Eighty microorganisms were isolated from twelve species of diseased fish (Table I).

TABLE I. Organisms isolated from diseased fish.

Species of fish	Disease symptom	Isolates
Abudefduf saxatilis	Skin lesions	*Micrococcus varians*
		Pseudomonas membranula (2 strains)
		Flavobacterium balustinum
		Unidentified species
	Fin-rot	*Micrococcus colpogenes*
		Aeromonas hydrophila
		Escherichia freundii
Tilapia mossambica	Eye-disease	*Pseudomonas aeruginosa*
		Pseudomonas reptilivora
		Pseudomonas ichthyodermis
		Achromobacter stenohalis
		Aeromonas hydrophila (2 strains)
		Bacillus filicolonicus
		Micrococcus colpogenes
	Fin-rot	*Pseudomonas aeruginosa*
		Pseudomonas ichthyodermis
		Bacillus megaterium
		Micrococcus colpogenes
		Flavobacterium marinovirosum
	Skin lesions	*Pseudomonas ichthyodermis*
		Escherichia freundii
		Alcaligenes faecalis
		Bacillus megaterium
	Gill-infection	*Pseudomonas ichthyodermis*
Therapon jarbua	Skin ailment	*Pseudomonas obscura*
		Achromobacter aquamarinus
Teuthis vermiculatus	Dropsy	*Pseudomonas species*
		Pseudomonas ichthyodermis (3 strains)
		Achromobacter species

(*Continued*)

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TABLE I. Organisms isolated from diseased fish. — (*Concluded*)

Species of fish	Disease symptom	Isolates
Coilia dussumieri	Eye blister	*Pseudomonas ichthyodermis*
Diagramma griseum	Eye disease	*Pseudomonas ichthyodermis*
Megalops cyprinoides	Fin-rot	*Pseudomonas ichthyodermis*
	Skin lesion	*Micrococcus candidus*
Drepane punctata	Skin lesion	*Pseudomonas ichthyodermis* (2 strains)
		Alcaligenes metalcaligenes
		Alcaligenes rectii
		Unidentified species
	Fin-rot	*Brevibacterium tegumenticola*
		Flavobacterium okeanoites
		Achromobacter stenohalis
Plotosus anguillaris	Muscle lesion	*Alcaligenes faecalis*
Scathophagus argus	Skin lesion	*Pseudomonas ichthyodermis*
		Achromobacter species
	Eye disease	*Pseudomonas ichthyodermis*
	"White spot"	*Achromobacter stenohalis*
	Fin-rot	*Flavobacterium okeanoites*
Trachynotus ovatus	Fin-rot	*Pseudomonas ichthyodermis* (2 strains)
		Flavobacterium piscida
		Flavobacterium dormitator
		Micrococcus colpogenes
	Skin lesion	*Achromobacter liquefaciens*
		Alcaligenes faecalis
		Alcaligenes marshalli (2 strains)
		Flavobacterium dormitator
		Pseudomonas ichthyodermis (3 strains)
		Micrococcus halodenitrificans
		Micrococcus colpogenes (2 strains)
	Eye disease	*Achromobacter liquefaciens* (2 strains)
	Upper lip lesion	*Flavobacterium suaveolens*
Mugil cunnesius	No visible symptoms	*Escherichia intermedia*
		Achromobacter species
		Aeromonas salmonicida (4 strains)
	Fin-rot	*Achromobacter aquamarinus*
		Achromobacter superficialis (2 strains)

The pathological materials used were scrapings of skin lesions, and diseased fins and scales. Specimens with inflamed and necrotic lesions under the skin were dissected in an inoculating chamber after the manner of Hunter (1920).

The media employed in the isolation of the etiological agents were fish infusion agar (100 ml of fish infusion,[1] 1 g peptone, 3 g NaCl, *p*H 7.4), nutrient

[1]For every gram of minced fish tissue 2 ml of distilled water was used for infusion overnight in a refrigerator. After heating for ½ hr in a water bath, the extract was strained and filtered and the filtrate utilized as fish infusion.

agar, and Sabouraud's agar. The temperature of incubation ranged from 27 to 30 C and *p*H of the medium from 7.4 to 7.6. The etiological agents were identified on the basis of their taxonomic treatment in Bergey's manual of determinative bacteriology (Breed et al., 1957) and ZoBell and Upham's monograph (1944).

In testing the pathogenicity of the isolates in fish, inoculum was introduced by each of two routes, the intramuscular and the intraperitoneal. Doses consisted of uniform saline suspensions of 24-hr-old cells. The suspensions were standardized with the second tube of the Brown turbidity series to contain 750–900 million cells per millilitre. The dosage varied depending on the route of inoculation. Through the intraperitoneal route the suspension was introduced in amounts ranging from 0.2 ml through 0.3 and 0.4 ml to 0.5 ml; through the intramuscular route, in graded amounts ranging from 0.1 ml to 0.3 ml. The intraperitoneal injection was done through the lateral aspect of the abdominal wall whereas the intramuscular injection was administered through the dorsolateral region just below the posterior end of the dorsal fin. Three sets of test fish, three per set, were employed for each dose, the fish being maintained under optimum conditions in an aquarium. One set of fish injected similarly with sterile saline served as controls. In all inoculations a 1-ml "tuberculin" syringe fitted with a fine bore needle, No. 25 or 26 gauge, was used.

In satisfying Koch's postulates, the pathogenicity studies were conducted with the two species of fish most easily available, *Tilapia mossambica* and *Abudefduf saxatilis*. Before inoculation the fish were anaesthetised with chloretone 1:2500.

Results and discussion.— Five isolates satisfied Koch's postulates. The other isolates failed to give rise to any definite disease symptoms for 8 days after injection.

Aeromonas salmonicida was able to set up a disease condition in *T. mossambica*. *Aeromonas hydrophila* produced disease conditions in both *T. mossambica* and *Abudefduf saxatilis*. *Pseudominas ichthyodermis*, *Alcaligenes faecalis*, and an unidentified species proved pathogenic to *A. saxatilis* only. The controls remained healthy. It was possible to reisolate all five pathogens.

Aeromonas salmonicida, which was isolated from *Mugil cunnesius*, is known to be the etiologic agent of a haemorrhagic septicemia or furunculosis (David, 1926; Belding, 1928; Davis, 1946). The disease was produced when the culture was administered intramuscularly, but not when it was introduced intraperitoneally. Horne (1928) found that *A. salmonicida* was lethal to goldfish when introduced intramuscularly, but not necessarily pathogenic when rubbed into a skin wound. Sixteen hours after the intramuscular injection the fish exhibited inflammation at the site of the injection. After 36–40 hr the body was discoloured with reddish haemorrhagic spots and patches, especially in the region of the caudal peduncle. The disease also spread to the fins, which "rotted" — the skin covering the fins was eroded and the naked fin-rays were exposed. When death appeared imminent the fish were dissected to study the internal picture, but no visible changes were found. The bacterium was

reisolated in pure culture. Throughout the investigation no furuncles were observed.

Aeromonas hydrophila, which was isolated from a diseased eye of *T. mossambica* and from fin-rot in *A. saxatilis*, produced disease in both species by both the intraperitoneal and the intramuscular routes. It was capable of killing both the test fish within 16–18 hr. The disease produced in the test fishes was a generalised nonhaemorrhagic septicemia with no external symptoms. The viscera were not affected.

Pseudomonas ichthyodermis, which was isolated from an eye blister on *Coilia dussumieri*, produced infection in *A. saxatilis* by both the experimental routes. Death of the host occurred within 36–44 hr. Discolouring and softening at the site of the inoculation were evident in 20–24 hr after inoculation. These symptoms were followed later by necrosis of the skin and inflammation of the underlying tissue muscles. In contrast with Hodgkiss and Shewan's findings (1950) no pus was produced.

Alcaligenes faecalis, which was isolated from a pyogenic lesion in *T. mossambica* and from a muscle lesion in *Plotosus anguillaris*, caused death of *A. saxatilis* within 40–48 hr. Slight inflammation occurred at the site of the inoculations but no other symptoms of a distinct nature were observed.

The unidentified species produced characteristic symptoms in *A. saxatilis* within 18 hr after injection. At the site of the injection there was a fading of the skin pigment, followed by inflammation and necrosis. At this stage the fish usually succumbed. The disease would often spread to the fins causing severe fin-rot, the caudal fin being reduced to a reddish stump. The pathogen, which was isolated from a skin lesion in *A. saxatilis*, was a Gram-negative, motile, non-sporogenous coccobacillus. It fermented glucose, sucrose, maltose, and mannitol, and gave positive reactions for the production of indole, catalase, and urease. Citrate was utilised as the sole source of carbon and H_2S was produced.

Conclusions.— The prominence being given to fisheries as a source of protein for human beings necessitates the study of fish diseases in order to select resistant species or varieties for pisciculture. Disease studies also help in the selection of suitable therapeutic substances, and experiments on the effects of antiseptics and antibiotics being conducted in this laboratory hold out hope of successful control and therapeutic measures for the fish diseases under study.

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Western Range Extension of the Surf Smelt, *Hypomesus pretiosus pretiosus*[1]

The surf smelt, *Hypomesus pretiosus pretiosus* (Girard), is common along the coast of British Columbia (Clemens and Wilby, 1961) and occurs as far south as Monterey Bay, California, but it has not been recorded farther west than Sitkalidak Strait in Alaska (McAllister, 1963). During a recent examination of the records of the Fisheries Research Institute's beach seine and surface trawl catches in Chignik Lagoon, on the south side of the Alaska Peninsula (56°20′N lat, 158°30′W long), we discovered two instances of capture of surf smelt. One was taken by beach seine on July 8, 1963 and another was captured by surface trawl on July 7, 1964. The fishing effort during the summers of 1963–67 consisted of 90 hauls with a 200-ft-long beach seine and 153 10-min tows by a surface trawl with a 6-ft^2 opening.

The former specimen (Fig. 1), an immature male, has been preserved in 10% formalin since capture and has now been placed in the fish museum,

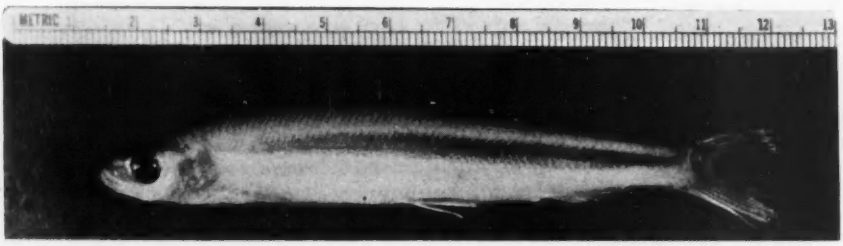

FIG. 1. *Hypomesus pretiosus pretiosus* (Girard) taken in Chignik Lagoon, Alaska (UW 19403).

University of Washington, Seattle, Wash. (Catalog No. UW 19403). The standard length of UW 19403 is 91.5 mm, the length of the longest pectoral fin ray is 12.4 mm, and the ratio of the longest pectoral fin ray to the distance between the insertions of the pectoral and pelvic fins is .4351. The pertinent meristic characters are 68 scales on the lateral line, 10+21 gill rakers on the first gill arch, 7 pyloric caeca, 10 dorsal fin rays, 20 caudal fin rays, 14 anal fin rays, 8 pelvic fin rays, and 15 pectoral fin rays. All measurements and counts were made from the left side, and according to the procedures established by Hubbs and Lagler (1958). Measurements were taken to the nearest 0.1 mm by vernier calipers.

Measurements and counts agree with those published by McAllister (1963), with the exception of the number of caudal fin rays, which McAllister listed as 19.

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UW 19403 is distinguished from the closely related *H. p. japonicus* (Brevoort) of the Asian coast of the Pacific Ocean by the number of scales on the lateral line (68), the number of pectoral fin rays (15), and the ratio of the longest pectoral fin ray to the distance between the insertions of the pectoral and pelvic fins (.4351). McAllister (1963) listed the ranges of these characteristics as 66–73, 14–17, and .41–.48, respectively, for *H. p. pretiosus*, and 63–67, 13–14, and .47–.55 for *H. p. japonicus*.

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Records of Earthworms from Vancouver, British Columbia

In a recent issue of this journal (Vol. 24, 1967, p. 1421), Mr W. P. Wickett reported on certain species of earthworms which he had found form an important constituent of the diet of fingerling coho salmon at Chef Creek, Vancouver Island. In the course of the report reference is made to a collection of earthworms made in and around Vancouver some 50 years ago by my late wife. It seemed of interest to compare those records from the mainland with these more recent ones from Vancouver Island and, fortunately, her notes on the collection are still in my possession. Dr Gates, of the University of Maine, Orono, Maine, had examined Mr Wickett's collection and I sent to him copies of my wife's notes. He was kind enough to check her identifications and to report on them and he has allowed me to quote the substance of his report *in extenso*.

"*Lumbricus herculeus* is of course *Lumbricus terrestris* which has not yet been correctly recorded in Canada west of Ontario (so far as publication is concerned). I have had a tail piece from British Columbia so I know the species still is there even in absence of any complete specimens. Your wife's record will be the first datum for the species in British Columbia. There is one record for Saskatchewan but the species involved actually was one of *Allolobophora*.

"*Lumbricus rubellus* was correctly identified and her record will be the first datum of that species for British Columbia. The species may prove to be the most common in British Columbia as I have had a considerable number of specimens from the province.

"*A. octoedra* is now as known *Dendrobaena octaedra*, and that probably was correctly identified. I have had specimens from British Columbia.

"*Dendrobaena rubida*, which I have also had from British Columbia, may have been the worm referred to on the last page, 'c', with clitellum in 26–31 and tubercula pubertatis in 28–30."

The species recorded by Wickett from Vancouver Island are not included in those found by my wife around Vancouver. All of them are, however, more or less cosmopolitan, so that the contention that all the earthworms of British Columbia are exotics is supported.

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